Hypothesis

A hypothesis for the possible involvement of microtubules and protein kinase in the mechanism of action of *cdc* 28 gene product of *Saccharomyces cerevisiae*

Anil Kumar Dudani and Rajendra Prasad

Membrane Biology Unit, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

Received 6 March 1984

Microtubule *Protein kinase* *Growth control* *G_1 arrest*
Cell cycle Saccharomyces cerevisiae

1. INTRODUCTION

A point of cell cycle regulation in the G_1 phase of *Saccharomyces cerevisiae* has been referred to as 'start'. [1,2]. This is the earliest known event in the yeast cell cycle and is mediated by the *cdc* 28 gene product [1,2]. Start is also the step sensitive to the presence of mating pheromone [1,2]. Cells that are below the critical size, are also arrested at or before the start event [3]. Before executing start, haploid yeast cells may either enter the stationary phase or undergo conjugation [3], but the onset of start commits cells to complete the mitotic cycle regardless of growth conditions [3]. Recent work indicates that start is the sole regulatory event for cell cycle initiation in yeast [4], and hence, efforts are underway to understand the molecular nature of start. In this hypothesis, an attempt has therefore been made to correlate the available data to suggest the possible mechanism of action of *cdc* 28 gene product.

2. THE *cdc* 28 GENE PRODUCT DEPENDENT FUNCTIONS

The earliest cell cycle event known to be blocked by cdc 28 mutation is the spindle pole body (SPB) duplication which precedes both initiation of DNA synthesis and binding [5], i.e., if this gene product fails to function, then the first step in the formation of the mitotic spindle, viz. SPB duplication, does not take place. Recent work on the cloning of *cdc* 28 gene suggests that this gene product may itself be involved in SPB duplication [5]. Piggott et al. [6] have suggested a bifunctional nature of *cdc* 28 gene product. They have shown that nuclear division (ND) is prevented by cdc 28-1N mutation but start function seems to be unaffected. Thus, *cdc* 28 gene product appears to affect directly or indirectly the mitotic spindle function. Furthermore, Prasad and his group recently suggested that *cdc* 28 gene product could also be involved in the overall metabolism of phosphatidylinositol (PI). They observed that G_1 arrest of cdc 28 cells was associated with a 35–40% drop in PI level, which recovered when cells were arrested past start [7,8]. It was shown that disfunction of mitotic spindle/microtubules, either by cdc 28 mutation or by some drug known to affect it, could also affect overall PI metabolism and that microtubules may be the possible potential candidate through which *cdc* 28 gene product elicits the observed effects [8]. Moreover, it was suggested that since start is the sole regulatory point in cell cycle initiation of *Saccharomyces cerevisiae,* the gene *cdc* 28 which mediates it could be pleiotropic [8]. All these observations suggest that the *cdc* 28 gene product may be inextricably associated with mitotic spin-

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

dle/microtubular function. In addition, by comparing the DNA sequence of *cdc* 28 gene with several viral oncogens, it was recently reported that *cdc* 28 gene product could be a protein kinase, as homology in DNA sequences was obtained with those viruses which have been demonstrated to encode protein kinases [9].

3. MICROTUBULE ASSEMBLY

It is known that tubulin is a phosphoprotein [10], has two binding sites for guanine nucleotides [11] and that the polymerization of tubulin requires nucleotide triphosphates. The existence of tubulin-associated protein kinase and phosphorylase activities led to the suggestion that phosphorylation and dephosphorylation of tubulin might play an important role in regulating microtubule assembly [11]. It has been observed that cyclic AMP could phosphorylate tubulin through its intrinsic protein kinase and that ATP as well as GTP could promote polymerization of microtubules in a low Ca^{2+} medium [12]. Vinblastine and vincristine at concentrations similar to those which promote selective aggregation of tubulin in vitro markedly increased the amount of ^{32}P transfer to tubulin [13]. It may, therefore, be concluded that the ability of tubulin to act as a substrate for phosphorylation is an important property and may serve to control the state of aggregation of microtubular proteins [13].

That *cdc* 28 gene product may be a protein kinase [9] and that mitotic spindle/micotubular function may be involved in *cdc* 28 gene action [7,8] and further that phosphorylation and dephosphorylation play an important role in microtubular aggregation [10–13] suggest that all these functions might be interrelated to elicit this gene action.

4. THE MODEL

We propose that *cdc* 28 gene product exhibits the observed effects through microtubular aggregation which in turn is under the control of protein kinase(s). If *cdc* 28 gene product is expressed as a protein kinase [9], it affects mitotic spindle/microtubular function, thus regulating different events such as traversal through G_1 by execution of start, nuclear division, PI metabolism. Failure of this gene product to function renders the cell incapable of accomplishing the above events. This model, however, suggests that a different protein kinase rather than the intrinsic protein kinase may be involved in microtubular aggregation but it certainly points towards a close link between microtubular status and protein kinase activity in *cdc* 28 gene product action. It is pertinent to mention here that protein kinases other than the intrinsic protein kinase could also be involved in phosphorylation of tubulin [14].

It has been shown that start can be executed even when the G_1 phase is absent and that the G_1 interval in yeast is only an interval of growth [15]. It is therefore tempting to conclude that since start is the sole regulatory point, *cdc* 28 gene product should have a pleiotropic effect. To accomplish this, cdc 28 gene product has to associate itself with a structure, through which it can have access to the entire cell. The microtubule is a potential candidate which qualifies in this respect, since it is not only present in the nucleus and cytoplasm, but also has interactions with the plasma membrane [16]. The gene *cdc* 28 can therefore control many processes by associating itself to mitotic spindle/microtubules. Future studies on protein kinase-dependent microtubular functions can shed some light on the regulation of cell cycle initiation in this simple eukaryotic organism.

REFERENCES

[1] Yanishevesky, R.M. and Stein, G.H. (1981) Int. Rev. Cytol. 69, 223–259.
[2] Hartwell, L.H. (1974) Bacteriol. Rev. 38, 164–198.
[3] Singer, R.A. and Johnston, G.C. (1982) in: Genetic Expression in the Cell Cycle (Padilla, G.M. and McCarty, K.S. ed) pp. 181–198, Academic Press, New York.
[4] Bedard, D.P., Singer, R.A. and Johnston, G.C. (1982) in: Genetic Expression in Cell Cycle (Padilla, G.M. and McCarty, K.S. eds) pp. 245–268, Academic Press, New York.
[5] Reed, S.I., Fergeuson, J. and Groppe, J.C. (1982) Mol. Cell. Biol. 2, 412–425.
[6] Piggott, J.R., Rai, R. and Carter, B.L.A. (1982) Nature 298, 391–393.
[7] Dudani, A.K., Trivedi, A. and Prasad, R. (1983) FEBS Lett. 153, 34–36.
[8] Dudani, A.K. and Prasad, R. (1984) FEBS Lett. 167, 151–154.

[9] Lorincz, A.T. and Reed, S.I. (1984) Nature 307, 183–185.
[10] Lagnado, J., Tan, L.P. and Reddington, M. (1975) Ann. NY Acad. Sci. 253, 577–597.
[11] Jacobs, M. (1975) Ann. NY Acad. Sci. 253, 573–576.
[12] Weisenberg, R.C. (1972) Science 177, 1104–1105.
[13] Lagnado, J., Tan, L.P. and Reddington, M. (1975) Ann. NY Acad. Sci. 253, 577–597.
[14] Rapperport, L., Leterrier, J.F. and Nunez, J. (1975) Ann. NY Acad. Sci. 253, 611–629.
[15] Singer, R.A. and Johnston, G.C. (1983) Exp. Cell Res. 149, 15–26.
[16] Dustin, P. (1978) in: Microtubules, pp. 79–114. Springer-Verlag, New York.