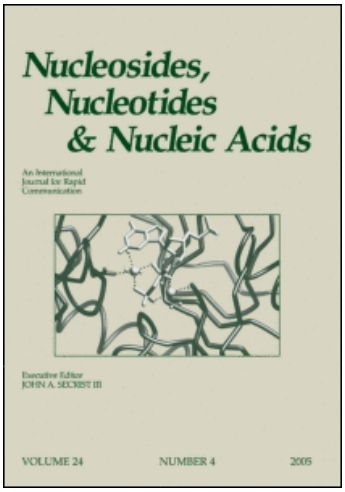

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Control of Differentiation by GTP

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CONTROL OF DIFFERENTIATION BY GTP

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ABSTRACT: A partial deficiency of GTP or GDP induces the differentiation of microorganisms and certain cells of higher organisms. The mechanism of this control may be retained during evolution.

Many cells respond to nutritional deficiencies by producing protective proteins, forming cysts or spores, or undergoing other types of differentiation. In yeasts and fungi, the cells first go through meiosis in order to generate recombinants that might be better adapted to the deprived environmental conditions before they produce new diploid cells. In higher organisms, differentiation starts during embryogenesis when some cells are no longer directly exposed to the environment bathing the fertilized egg. Only later in development is differentiation controlled by hormones; whether other types of differentiation are then still determined by the nutritional supply of cells is not known but likely. Presumably, cells have retained during evolution the signal mechanisms initiating differentiation and continue to use them at various stages of development. I want to demonstrate this for several cell types.

Microbial differentiation generally starts when an essential nutrient becomes growth rate limiting. The starvation must be slow and not abruptly complete because otherwise all metabolism stops and differentiation, which requires the synthesis of new macromolecules, cannot occur. Because the ability to differentiate is important, some organisms have developed means to accumulate, often toward the end of exponential growth, polymers that can later be slowly degraded and used as nutrients when the cell senses a nutritional deficiency[1].

When the same type of differentiation results from the starvation of any one of different nutrients, the cell presumably recognizes the change of the same ultimate signal compound. Using sporulation of Bacillus subtilis as tool, this signal compound has been identified in my laboratory as GDP or GTP[2]. These two nucleotides decrease under all sporulation conditions whereas other compounds increase or decrease depending on the particular condition used. Conversely, one

can efficiently induce sporulation by specific inhibitors of GTP synthesis such as mycophenolate (inhibits IMP dehydrogenase) or decoyinine (inhibits GMP synthetase) when they are used at concentrations at which they inhibit nucleic acid synthesis only little[3]. Coadditon of guanine or guanosine prevents this induction. To avoid the argument that inhibitors often affect more than one reaction, it has also been shown that guanine starvation of a (leaky) mutant deficient in GMP synthetase induces sporulation. The possibility that GMP (rather than GDP or GTP) controls differentiation was excluded by the use of formycin-A; it induces sporulation and causes a decrease of GDP and GTP but an increase of GMP[4]. In the following, when I say that differentiation is caused by a decrease of GTP, I mean GTP or GDP.

Meiosis and sporulation in the yeast Saccharomyces cerevisiae is also induced by nutrient starvation which always results in a decrease of guanine nucleotides. It can also be specifically induced by inhibitors of GMP synthesis, such as mycophenolate or virazole, or by guanine starvation of a guanine auxotroph. Interestingly, adenine powerfully inhibits the uptake or conversion of guanine to GMP in yeast and thereby causes, in the presence of 100 uM guanine, the deprivation of guanine nucleotides in the guanine auxotroph, resulting in efficient sporulation[5]. Other small molecular weight compounds such as other nucleotides or S-adenosyl-methionine have been excluded as the ultimate signal.

GTP is used in numerous enzymatic reactions as substrate or cofactor. In considering which of these might be involved, the following observations are important:

1. Sporulation is efficiently induced when the intracellular concentration of GTP decreases from 600 to 100 uM. The latter is still much higher than the K_m of many GTP requiring enzymes. It is therefore unlikely that sporulation is controlled by compounds (ppGpp, G-proteins, or proteins involved in protein synthesis) whose synthesis or function optimally requires only a few uM or less GTP.
2. B. subtilis has no detectable cAMP and at most 6 nM cGMP[6,7]. It is unlikely that these two compounds have anything to do with the control of differentiation by GTP if one considers it likely that the signal mechanism whereby GTP controls differentiation is similar in all organisms. Yeast produces significant amounts of cAMP which change under various conditions. But it has been shown for a guanine mutant that neither a decrease nor an increase in cAMP are important for sporulation[8].

Similar studies with a human glioma cell line (NHG1) have shown that mycophenolate can induce the synthesis of the intermediate filament protein called glial fibrillary acidic protein (GFAP) which is only found in astrocytes[9]. The effect was optimal at an intermediate mycophenolate concentration (5 uM). At that concentration, the overall rate of RNA synthesis was not affected and that of

protein synthesis decreased only transiently; but, as is typical for committed differentiating cells, the number of cells no longer increased. Using labeled cDNA for the GFAP gene, it was shown that during the induction by mycophenolate the concentration of GFAP mRNA increased 15 fold and that isolated nuclei continued to synthesize new GFAP mRNA; thus the control is at the transcription level. The addition of guanosine (100 uM) prevented the induction of GFAP and the inhibition of growth (Lipsky and Freese, in preparation).

Differentiation has also been induced by the deprivation of guanine nucleotides in a myeloid cell line. During hematopoiesis early stem cells differentiate into lymphoid and myeloid stem cells which in turn give rise to numerous other cells lines including erythrocytes, granulocytes, macrophages and platelets. This differentiation is normally controlled by "colony stimulating factors" (CSF), many of which have now been isolated[10]. One of the intermediate cell types (GM) was found arrested in its development in a leukemia cell line (HL60)[11]. But the cells could be caused to enter the neutrophil differentiation path, which leads to the formation of granulocytes, by retinoic acid (1 uM) and some other agents. Wright and collaborators found that under these inducing conditions IMP dehydrogenase and consequently GTP decreased; the induction could be specifically prevented by the addition of guanosine[12]. Golub (personal communication) has explained this effect by showing that retinoate inhibits the flow of electrons to diferric transferrin, thereby decreasing the concentration of NAD which is essential in the reaction of IMP dehydrogenase converting IMP to XMP. Thus the effect of retinoate is indirectly similar to that of mycophenolate. Lucas et al. further demonstrated that mycophenolate and other inhibitors of GMP synthesis (3-deazaguanosine, thiazofurine and its seleno-analog) also induce the maturation of HL60 cells into neutrophils[13]. These studies show clearly that cancer cells are not locked irreversibly in an immature state, in which they proliferate indefinitely, but can be induced to differentiate by means of the correct agent. They simply have lost the response to the normal inducing agent (e.g. a CFU), e.g. by no longer making the necessary receptor. This knowledge is now used in the attempt to treat some cancers, especially leukemias, by inhibitors of GTP synthesis used at subtoxic concentrations [14].

Conclusion: The concentration of GTP (or GDP) controls the onset of differentiation of rather different cell types. Presumably, more such responding cell types will be found in both microorganisms and higher organisms because this control of differentiation was presumably retained during evolution. The understanding of this phenomenon has medical consequences because one may be able to control certain cancers or diseases of the immune system by promoting or avoiding the maturation of differentiating cells. In addition, the control of microbial or plant differentiation or of individual compounds, such as antibiotics,

associated with this process will have agricultural and industrial consequences. Thus it is clearly worthwhile to work out the molecular mechanism of this differentiation control.

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