tasch, S. *Astron. Astrophys.* **94,** 213; 1981), but the stars themselves are not directly examined. Satellite observations in the near ultraviolet indicate some extreme temperatures, but these result from observational error and difficulty in understanding the nature of the radiating stellar atmospheres. Atherton *et al.* (*Astrophys. J.* **232,** 786; 1979) located what may be a star of more than 200,000 K in a nebula similar to NGC2440, NGC7027, but the identification is only tentative.

We see then the significance of the new work by Atherton and colleagues — for the first time such a hot star has been unambiguously seen, and its temperature measured. Now the wall has been truly broken, pioneering the technique necessary to fill in one of the few remaining holes in the paths of stellar evolution. □

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**Intracellular signalling**

# GTP and calcium release

*from P.F. Baker*

CALCIUM is an important intracellular signal, key features of the system being the maintenance of a low concentration of free Ca in the cytosol, usually in the region of 100 nM; the existence of pools of Ca, both intracellular and extracellular, at much higher concentrations; mechanisms for transferring Ca from these pools into the cytosol; and, finally, receptors in the cytosol that can respond to changes in free Ca in the 100–1,000 nM range. Although much is known about these receptors and how the various pools of Ca are established, surprisingly little is known about how Ca is permitted to move downhill from these pools into the cytosol. At the plasma membrane the problem seems to be solved by the existence of Ca channels that are gated either chemically or by voltage, and it has always seemed likely that something similar may exist to effect intracellular release; but only recently have possible mechanisms begun to emerge, one of which is presented by Gill *et al.* on page 461 of this issue[1].

The two major intracellular organelles that bind Ca are the mitochondria and the endoplasmic reticulum. Although their relative importance has long been debated, largely through the application of X-ray microanalysis to fast-frozen tissue, there now seems general agreement that in resting cells mitochondria contain rather little Ca but can sequester massive amounts should the cytosolic Ca begin to rise. In contrast, despite its relatively small Ca-binding capacity, the endoplasmic reticulum looks the stronger candidate for a high-affinity, physiologically relevant, intracellular Ca store. This comes as no surprise to muscle physiologists, who have long recognized that the myofilaments of skeletal and cardiac muscle are surrounded by a highly specialized derivative of the endoplasmic reticulum — the sarcoplasmic reticulum, which serves to regulate the local Ca concentration to which the Ca-sensitive contractile apparatus is exposed. Even in this tissue, however, which is apparently ideal for experimental investigation, there is still considerable uncertainty about how Ca is released. Likely mechanisms for coupling electrical excitation to Ca release continue to be dominated by variations of the electro-mechanical, charged-plug model first put forward more than 10 years ago by Schneider and Chandler[2].

The rise to prominence of the endoplasmic reticulum has brought new ideas about Ca mobilization and there now seems to be more known about the mechanism of Ca release from this structure than from the sarcoplasmic reticulum. A major step forward was the discovery by Streb *et al.*[3], rapidly confirmed in many laboratories, that the water-soluble molecule inositol trisphosphate ($IP_3$), a hydrolysis product of the membrane lipid phosphatidylinositol bisphosphate, can release Ca from the endoplasmic reticulum of many cells. In one step, this provided a link between membrane receptors and release of Ca from a major intracellular store. All subsequent work points to the existence in the endoplasmic reticulum of some sort of $IP_3$-gated calcium channel. The obvious and very interesting possibility that $IP_3$ may also be involved in releasing Ca from the sarcoplasmic reticulum of skeletal muscle (see the recent discussion in these columns[4]) received some early and dramatic support but has so far failed to gain general acceptance. A subtle variant however, such as an $IP_3$-type molecule as part of the Schneider–Chandler plug acting to release Ca via an $IP_3$-type receptor in the sarcoplasmic reticulum is still an open possibility. In addition, by the isolation of nucleotide-gated channels, Smith *et al.*[5] have given new impetus to the search for physiologically relevant, chemically gated Ca channels in the sacroplasmic reticulum.

Now, Gill *et al.*[1] interpret some very interesting new data in terms of another mechanism. Working with a detergent-permeabilized neuroblastoma cell line, they report that approximately half of the Ca accumulated by a store, presumed to be the endoplasmic reticulum, can be released by exposure to micromolar concentrations of GTP. This very clear effect is highly specific for GTP and cannot be mimicked by non-hydrolysable analogues suggesting that GTP hydrolysis might be an essential part of the Ca-release process. GTP-dependent release is temperature-dependent and can be inhibited competitively by GDP. The authors make a plausible case for the involvement of a GTP cycle in the control of calcium permeability in the endoplasmic reticulum of neuroblastoma cells. They fail to explain, however, why their system is not permanently activated by the levels of GTP always present inside these cells.

What is the relation between GTP-induced and $IP_3$-induced Ca release? The authors claim they are two separate systems. Thus, although $IP_3$ is active in their cells, its effects are not inhibited by GDP and exhibit a different temperature dependence from GTP-induced release. Although suggestive, these arguments are not compelling and, as the authors admit, it is possible that the $IP_3$- and GTP-dependent systems for releasing Ca may prove to be closely related. The finding of Dawson[6] that $IP_3$-induced release is strongly stimulated by GTP might be highly significant in this respect. GTP-binding proteins are fast coming to occupy a central position in coupling membrane events and their classic role in coupling occupied receptors to cyclase activation now finds parallels in the activation of phospholipase C and even the opening of channels[7,8], so there is ample precedent for a similar role in effecting Ca release from the endoplasmic reticulum. According to this view, the preparation of Gill *et al.* may contain an endogenous ligand that can, in the presence of GTP, open Ca channels in the endoplasmic reticulum. The requirement for both GTP and an unknown ligand would both circumvent the tricky question of why physiological levels of GTP do not keep the channels open permanently and focus attention on the nature of the hypothetical intracellular ligand, the binding of which is coupled by GTP to channel opening. If such a ligand exists, it could still be $IP_3$ or a close relative. The onus seems to be on Gill and his colleagues to prove it does not exist. □

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