REVIEW ARTICLE

What makes *Candida albicans* pathogenic?

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***Candida albicans* is an opportunistic pathogen of human beings and other mammals. Two other features, besides its pathogenicity, have made it a popular organism of study. It exists in different cellular forms and can change from one form to another, depending on growth conditions. Thus, it is being used as a model system to study cellular differentiation. It can also heritably and reversibly switch its cellular and colony morphologies.**

Candida albicans, a pathogenic dimorphic yeast, is frequently found as a commensal on the oral, gastrointestinal, and vaginal mucosae in humans and other warm-blooded animals. It causes a variety of clinical forms of illness ranging from localized cutaneous candidiasis in healthy individuals to life-threatening systemic candidiasis in immunocompromised hosts. An important feature of *C. albicans* pathogenesis is that when the host defence mechanism breaks down it becomes infectious resulting in candidiasis. Host defence mechanisms to *C. albicans* infections are skin-mucosal, barriers, immune system and hormone levels. Breakdown of any of these defence mechanisms predisposes the host to *C. albicans* infections[1,2]. The last two decades have seen an increase in the incidence of candidiasis, which is attributed to the widespread use of antibiotics and immunosuppressive drugs. The increase in the number of acquired immunodeficiency syndrome cases is also responsible for the higher incidence of candidiasis. *C. albicans* is therefore considered the most common opportunistic human pathogen.

Do mycelia matter?

Morphogenesis of *C. albicans* has attracted particular attention, since it is relevant to the virulence of the organism[1]. It also provides a good model system to study eurkaryotic cell differentiation. In the last few years, several reviews have been published on the biology, genetics, pathogenicity, and morphogenesis of *C. albicans*[1,2,3]. *C. albicans* is classified as Fungi Imperfecti because it lacks a known sexual stage in its life cycle. It exists in different cellular morphologies. The most commonly encountered growth form of *C. albicans* is as a budding yeast (Figure 1). It develops through a mitotic life cycle, $G_1 \longrightarrow S \longrightarrow G_2 \longrightarrow M$, in the same way as other yeasts, but it has proven extremely difficult to define these phases clearly in *C. albicans*[1]. Depending on environmental factors, budding cells can give rise to true hyphae or pseudohyphae. The hyphae grow continuously by apical extension. Mitotic nuclear division occurs within an extending hypha, and septa are formed. The germ tube, an intermediate stage in yeast to hypha transition, is a newly evaginating hypha till the formation of the first septum. Hyphae may give rise to blastospores,

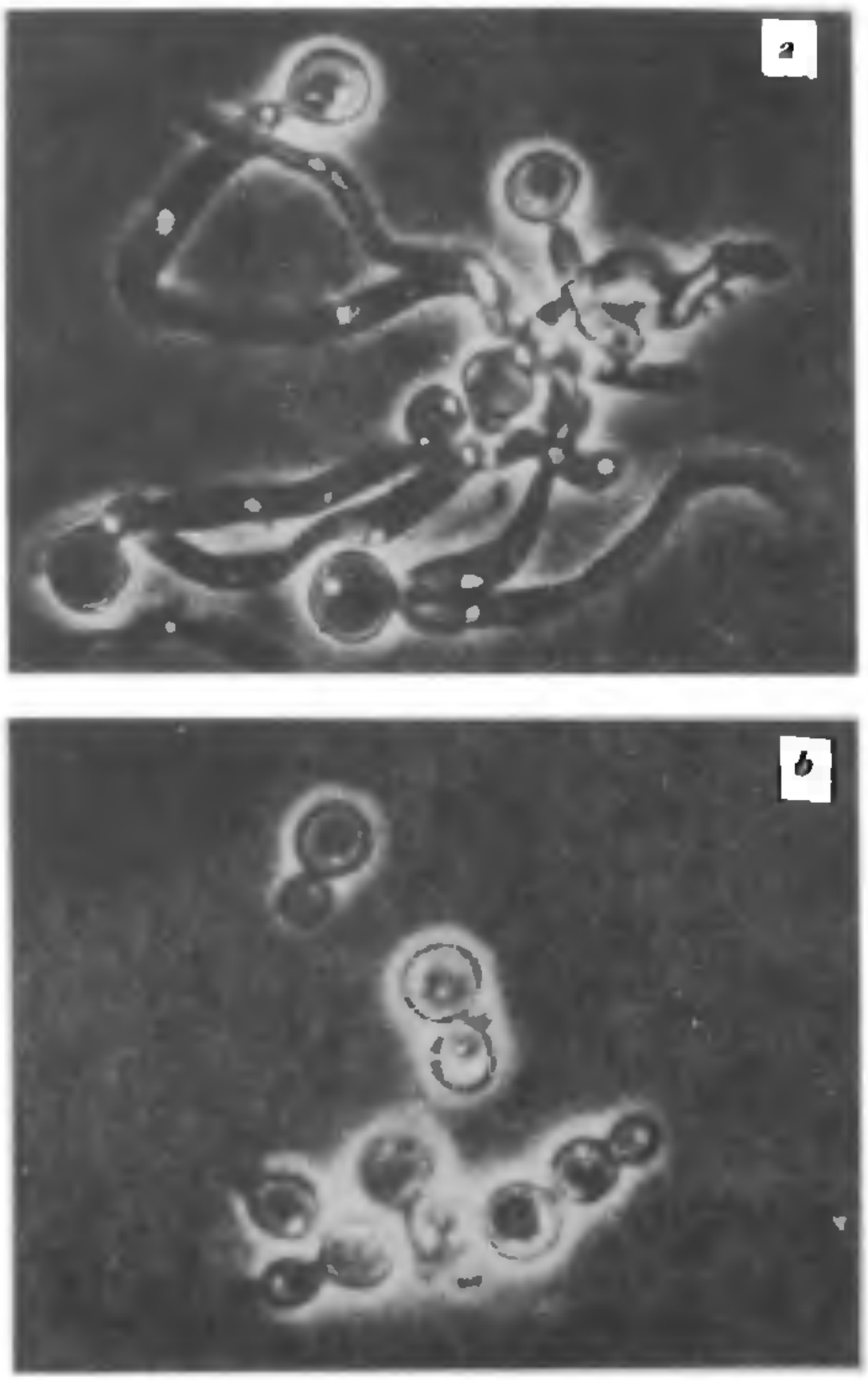

Figure 1. Various morphological forms of *C. albicans* as viewed by phase-contract microscopy. *a*, hyphae; *b*, buds.

which bud off laterally. Pseudohyphae are elongated yeast cells formed by polar budding, constricted at cell junctions, and usually joined in chains or clusters. Mycelium is the entire fungal cellular aggregate, including the hyphae and the buds. The change from yeast to hyphal form is referred to as dimorphic transition and can be induced by a number of factors[1].

Another interesting feature of *C. albicans* is its ability to switch colony morphologies. It switches heritably, reversibly, and at a high frequency (10^{-2} to 10^{-4}) among at least seven colony phenotypes[4] or between white and opaque color phenotypes[5]. Colony morphology switching may cause a variation in susceptibility to drug and hence in the pathogenicity of the organism.

Dimorphism in *C. albicans* is regulated by a wide variety of environmental factors which include temperature, pH and nutrients[1,2,3]. A temperature range of 33 to 42°C and a pH range from 6 to 8 are critical for germ tube formation. However, it has been shown that, under certain conditions, cells can form germ tubes at 25°C[6] and at a pH as low as 3.0 (ref. 7). Furthermore, germ tube formation in defined media is also influenced by yeast growth phase and by strain variation[1,2].

Several experimental approaches have been used to investigate morphogenesis in *C. albicans*. Many studies have been carried out to identify the biochemical and molecular events responsible for morphogenesis[1,3], but the underlying mechanism has not been clear. RNA and DNA syntheses during morphogenesis have been studied[8]. A number of workers have examined protein profiles by both one- and two-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis during morphogenesis in *C. albicans*[1], but proteins specific to either yeast or mycelial form have not yet been identified. Unfortunately, the media and growth conditions used in these studies were often highly complex and very different. Furthermore, *C. albicans* strains display a great deal of heterogeneity in germ tube and mycelium formation[2]. In addition, the diversity of environmental factors which regulate morphogenesis has made it difficult to find a unifying explanation for morphogenesis in *C. albicans*.

Compared with yeast forms, hyphal forms contain more chitin[9,10] and possess higher chitin synthase activity[11]. Studies with inhibitors have shown that chitin synthase can play a regulatory role in morphogenesis of *C. albicans*[12]. It has been shown that chitin synthase plays a fundamental role in morphogenesis of *Saccharomyces cerevisiae*[13].

Actin granules also appear to be associated with the growth zones of yeast and hypha of *C. albicans*[14]. There is a difference in actin localization during formation of buds and hyphae. In budding cells, actin granules are distributed throughout the cytoplasmic cortex, while during hyphal growth the majority of the actin granules are clustered at the apex. Recently, we have shown that overexpression of actin gene is associated with the morphogenesis of *C. albicans*[15]. In addition to actin, atleast two other genes encoding 50 kDa and 30 kDa proteins are also overexpressed.

One of the possible ways by which fungi can respond to diverse stimuli and undergo dimorphic transition is through involvement of a second messenger such as cyclic AMP (cAMP) or Ca^{2+}. There are contradictory reports about the involvement of cAMP in the regulation of morphogenesis in *C. albicans*[16–18], though both cAMP-dependent and independent protein kinases are present[19,20]. Ca^{2+} is known to be involved in growth and differentiation of many fungi. Its action is mediated mainly through the binding of calmodulin, a ubiquitous Ca^{2+} binding protein that regulates many cellular processes in eukaryotes. Using several calmodulin inhibitors, it has been shown that germ tube (an intermediate stage of yeast and hyphae) formation depends on the activity of calmodulin[21]. Recently, calmodulin has been purified from *C. albicans* and results from our laboratory indicate a possible involvement of Ca^{2+} and calmodulin in germ tube formation of *C. albicans*[22]. Activation of cell surface receptors in response to environmental factors may increase the cytoplasmic concentration of Ca^{2+} and hence the initiation of Ca^{2+}-dependent events. Increased intracellular Ca^{2+} levels activate calmodulin, which can be considered as a primary event that triggers a series of biochemical changes, eventually leading to morphogenesis. Ca^{2+} and calmodulin may regulate morphogenesis in *C. albicans* by affecting phosphorylation of specific proteins, by regulating cAMP levels, or by modulating synthesis, localization or polymerization of actin and microtubule organization or chitin synthase activity (Figure 2). In *C. albicans*, germ tube formation is accompanied by an increase in cytoplasmic pH from 6.8 to 8.0 (ref. 23). It has been shown that the amount of Ca^{2+} complexed to calmodulin varies by a factor of 10 over a pH range of 6.5 to 7.5 (ref. 24). It is therefore resonable to think that changes in intracellular pH can mediate regulation of morphogenesis by altering calmodulin activity.

Phosphorylation of proteins is a key step in the regulation of cellular metabolism. In *C. albicans*, germ tube formation is accompanied by an increase in rate of protein phosphorylation[21]. Recently, our results indicate that germ tube-specific phosphoproteins are phosphorylated in germ tube-forming cells and dephosphorylated in budding cells. Similarly, bud-specific phosphoproteins are relatively more phosphorylated in bud-forming populations and dephosphorylated in the germ tube-forming cells. Thus, morphogenesis in *C. albicans* could be regulated by phosphorylation and dephosphorylation of specific proteins. Characterization of these morphology-specific phosphoproteins and identification of the putative protein kinases might unravel the signal-trans-

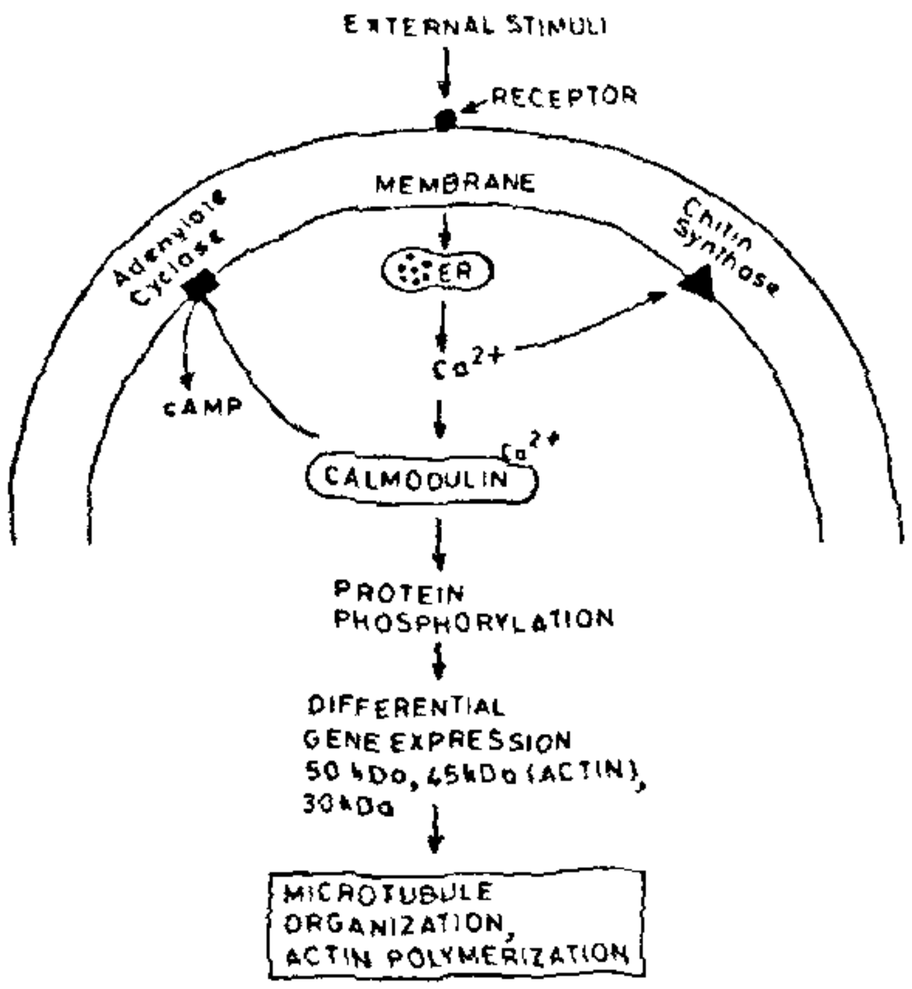

Figure 2. *Postulated physiological function of Ca^{2+} and calmodulin in morphogenesis of C. albicans.*

duction pathway involved in morphogenesis. Furthermore, if genes coding for these proteins are cloned, their control of expression can be studied. From such studies, it would be possible to know more about, and finally to identify the master control gene which determines differentiation and pathogenesis[25].

Is secretory acid proteinase a virulence factor?

Ability to form hyphae, adherence to epithelial surfaces, and secretion of hydrolytic enzymes appear to be important for virulence of *C. albicans*[1]. The ability of *Candida* species to adhere specifically to host surfaces is a pre-requisite for invasion.

Recently, a lot of attention has been focused on secretory acid proteinase, though *Candida* secretes other hydrolytic enzymes e.g. phospholipase, *N*-acetylglucosaminidase[1,3]. Indirect evidences supporting *C. albicans* proteinase as a virulence factor are summarized as follows. The enzyme has been shown by immunofluorescence to be synthesized in infected tissues *in vivo* and antibodies to the purified enzyme are found predominantly in patients with visceral *Candida infections*[26–28]. Furthermore, it was shown that a mutant with a defective or impaired proteinase secretion was less virulent for mice than their fully proteolytic parental organisms[27,29]. Ghannoum and Elteen[30] reported that the ability of *C. albicans* to secrete proteinase could be correlated to adherence capacity and tissue colonization. Furthermore, we have also established a good correlation between proteinase production and pathogenicity of various species of *Candida*[31]. To understand molecular details of the process of induction as well as secretion and to study its role in pathogenesis, recently we have cloned the *proteinase gene*[32].

Why study aminosugar metabolism?

Aminosugars are present in mucous membranes, which are the sites of colonization by *C. albicans*. Since *C. albicans* has to survive on the mucous membrane by utilizing a sugar (possibly aminosugars) as a source of energy, we initiated studies on aminosugar metabolism in *C. albicans* to gain a better understanding of the biochemical basis of candidiasis. A comparative study of utilization of *N*-acetylglucosamine (GlcNAc) by pathogenic and non-pathogenic strains was carried out in our laboratory[33]. Interestingly, non-pathogenic yeasts cannot utilize GlcNAc, which suggests that the aminosugar metabolic pathway is important in pathogenesis.

The aminosugar metabolic pathway in *C. albicans* was first elucidated in our laboratory. *N*-acetylglucosamine is transported by a membrane-associated permease[33–36]. The transported sugar is metabolized by the sequential action of GlcNAc kinase[37–41], GlcNAc 6-phosphate deacetylase[42,43] and glucosamine 6-phosphate deaminase[44,45]. All these enzymes are induced on addition of GlcNAc to cultures of *C. albicans*. A typical feature of this system is the absence of glucose repression[46,47]. Western blot analysis revealed that the deaminase level was stimulated 20-fold when GlcNAc was added to a growing glucose-containing culture of *C. albicans* (Natarajan and Datta, unpublished result).

N-acetylmannosamine (ManNAc) induces ManNAc 2-epimerase activity in *C. albicans*[48,49]. Moreover, ManNAc induces all of the enzymes of GlcNAc metabolism[48–50]. Glucosamine (GlcN) also supports the growth of *C. albicans*[51]. This sugar is transported probably by a general sugar permease, and a GlcN kinase is not induced by GlcN[51–53]. The aminosugar metabolic pathway is summarized in Figure 3. Presently, it is speculative whether the capability to metabolize GlcNAc is important for pathogenesis because the sugar in the free form is not available at the site of colonization. However, *N*-acetylglucosamindase (chitobiase), which is also induced by GlcNAc[54], might release GlcNAc residues from glycoproteins. These residues can be utilized by *C. albicans* for it's growth.

The inducible GlcNAc-metabolizing system is a good model to study the regulatory circuits of gene expression. Studies with inhibitors indicate that the genes of the GlcNAc metabolic pathway are regulated at the level of transcription[2]. Recently, we have cloned glucosamine-6-phosphate deaminase gene and confirmed

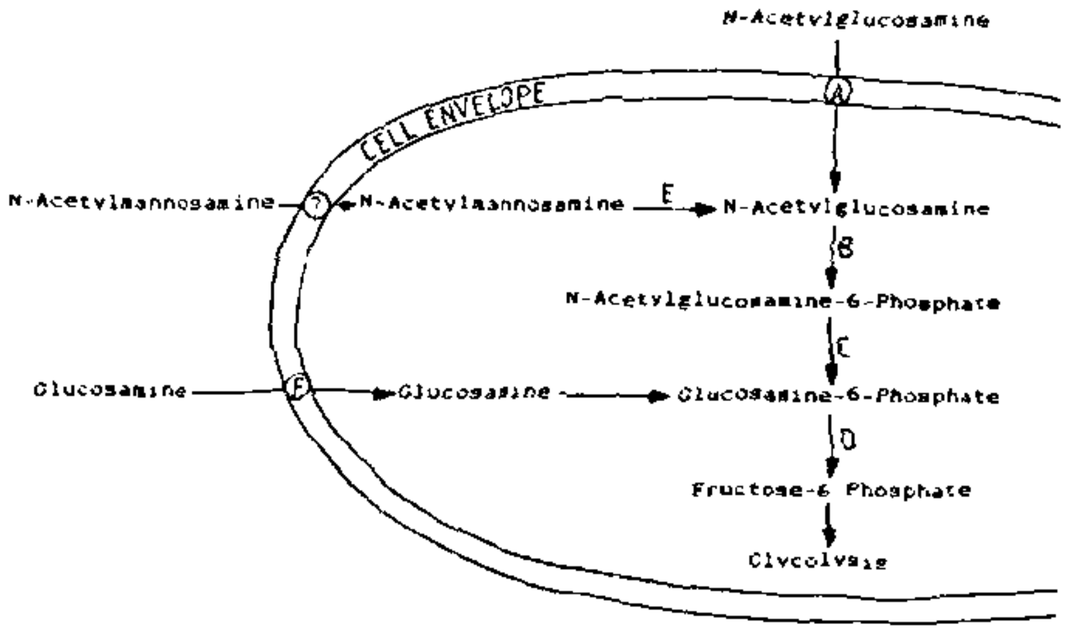

Figure 3. Aminosugar metabolism in *Candida albicans*; ***A***, *N*-acetylglucosamine permease, ***B***, *N*-acetylglucosamine kinase ***C***, *N*-acetylglucosamine 6-phosphate deacetylase, ***D***, glucosamine 6-phosphate deaminase, ***E***, *N*-acetylmannosamine 2-epimerase, ***F***, general sugar permease. *Candida albicans strains seem to vary in their capacity for N*-acetylmannosamine transport.

that genes of *N*-acetylglucosamine catabolic pathway are not linked and regulation occurs at the level of transcription (Natarajan, K and Datta, A, manuscript in preparation). Molecular studies of this inducible pathway are important for the following reasons: (i) it can serve as a model system to study genetic regulatory circuits controlling gene expression in eukaryotes. (ii) *C. albicans* is a medically important organism due to its pathogenicity. Several groups are attempting to understand the mechanism of *C. albicans* virulence. Molecular approaches would be useful to understand virulence and (iii) since aminosugar utilization is an attribute of pathogenic yeasts it is imperative to study in detail the fundamental aspects of gene regulation.

Overall comments

C. albicans is naturally diploid and no sexual cycle has been found. The base composition of the genome is about 35% *G*+*C* and the DNA content per cell (diploid) is about 37 femtograms[55]. Magee *et al.*[56] have provided evidence for the presence of seven chromosomes in *C. albicans*. Since *C. albicans* is diploid and asporogenous, mutants are difficult to obtain. However, if cloned genes are available, mutant strains can be constructed by *in vitro* gene disruption and directed mutagenesis.

Most of the cloned *C. albicans* genes have been isolated through their counterparts in *S. cerevisiae*. Several approaches have been made including complementation, sequence homology and the ability of certain *C. albicans* sequences to confer new phenotypes on *Saccharomyces* strains. Genes cloned in this fashion range from the highly conserved actin[57] and tubulin[58] genes to those involved in aminoacid biosynthesis[59] and sugar utilization[56].

The directed mutagenesis technique should facilitate construction of stable mutants. If aminosugar metabolism or proteinase secretion is necessary for *C. albicans* infection, mutants are required. Towards this end, we have cloned genes encoding secretory acid proteinase and glucosamine-6-phosphate deaminase, the terminal enzyme of the *N*-acetylglucosamine catabolic pathway. Further studies directed towards raising the stable mutants by directed mutagenesis are likely to be challenging and rewarding.

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RESEARCH ARTICLES

Magnetic dynamics in La_2CuO_4

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A coherent account of the magnetic properties of La_2CuO_4 and other cuprates providing a quantitative understanding of the magnetic dynamics in both the ordered as well as disordered states, is presented within the framework of a generalized spin-wave theory applied in conjunction with scaling ideas. The unique qualitative features—the essentially linear falloff of the sublattice magnetization with temperature in the ordered state, the exponential temperature dependence of the spin correlation length in the disordered state, and the characteristic energy dependence of the integrated structure factor—result from the long-wavelength spin-wave dynamics in two dimensions.

THE remarkable manifestation of the almost two-dimensional, spin-$\frac{1}{2}$ antiferromagnetism in La_2CuO_4 and similar cuprates has led to a quantitative understanding of the effects of quantum and thermal spin fluctuations, while providing sustained impetus in efforts to understand low-dimensional, low-spin magnetism. The discoveries of long-range AF order[1], and long-range spin correlations above the Néel temperature[2], have helped clarify important theoretical issues. Most significantly, for the spin-$\frac{1}{2}$ antiferromagnet on a square lattice, it became quite clear that quantum spin fluctuations, although appreciable, are not strong enough to destabilize the Néel state, in favour of spin liquid-(RVB) type states[3], and that the Néel state is robust even for small frustration of next-nearest coupling[4].

La_2CuO_4 and other related cuprates are highly layered systems, characterized by extremely weak interlayer magnetic coupling, responsible for the 3D AF ordering below the Néel temperature. This coupling actually depends, in a very subtle manner, on details of their structural characteristics. In La_2CuO_4 the effective exchange coupling between Cu spins in neighbouring layers is due to the orthorhombic distortion which