Yeast Cell Envelopes: Biochemistry, Biophysics, and Ultrastructure

Volume I

Editor

Wilfred Niels Arnold, Ph.D.

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PREFACE

Yeasts hold important associations with man in at least three arenas. As industrial agents their positions in the fermentative and baking industries are household knowledge. A few yeast or yeast-like species are pathogenic. And, as experimental eukaryotic cells, yeasts continue their long history as useful subjects for studies on metabolism, genetics, and molecular biology.

A comprehensive review of the yeast cell envelope has not appeared previously and we trust that this attempt will be timely. The title of this volume was chosen to reflect the three major areas of contribution to our current understanding of the cell envelope, but we have not attempted to group chapters into subdivisions. That would be somewhat arbitrary at best. In fact, the contributing authors were recruited for their interdisciplinary work as well as their special expertise.

The approach is to describe phenomena, to review the literature, and to illuminate outstanding problems. We have also attempted to generate working hypotheses which may stimulate further studies. That some of these ideas be of germinal value is of more concern to us than that all of the hypotheses should stand the test of further experimentation.

Brenda Johnson has given special assistance in the assemblage of this volume. I also wish to acknowledge my former teachers and mentors Drs. G. Langdon, J. Middleton, J. Bald, and J. Thompson, as well as two colleagues, E. Juni and I. Goldstein, for encouragement during my formative years in biological chemistry.

W. N. Arnold
Westwood Hills, Kansas
July 1980

THE EDITOR

Wilfred N. Arnold, Ph.D., is Professor of Biochemistry at the University of Kansas Medical Center, Kansas City, Kansas. Dr. Arnold received a B.S. from the University of Queensland, Australia in 1956, an M.A. from the University of California, Los Angeles in 1958, and a Ph.D. from Cornell University, Ithaca, N.Y. in 1962. He has held postdoctoral fellowships at the Waite Institute, University of Adelaide, Australia and at the University of Wisconsin Medical School, Madison. Before coming to Kansas in 1971, he held research positions at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia and the University of California at Riverside; and an Assistant Professorship at Wayne State University Medical School, Detroit, Mich.

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Chapter 1

INTRODUCTION

W. N. Arnold

A working definition of the class of organisms collectively known as "yeasts" is not easily postulated. They are unicellular, eukaryotic microorganisms that typically reproduce by budding. So much is true for the majority of yeasts but one soon runs into exceptions; there are species with a propensity for mycelium formation, others with a fission type of cell division, and still another genus that bears conidia on sterigmata. As discussed by Lodder[1] (Table 1) the term "yeasts" developed historically and really includes a heterogeneous group of microorganisms. Phaff et al.,[2] have reviewed the etymology of "yeast", and equivalent terms in other languages, and point out the consistent relationship to fermentation. However, they also conclude that the name embraces a heterogeneous collection of fungi.

The number of defined yeast species is now somewhat in excess of 500. In addition there are a number of fungi with "yeast-like" cells as part of their cell cycle. At the other extreme some biochemists incorrectly use the term yeast as synonymous with *Saccharomyces cerevisiae*. One of the themes that will emerge in the following chapters is that a rich variety of additional species awaits further biological investigation and that in some circumstances the more exotic species may offer advantages for experimental study.

This book is devoted to the yeast cell envelope. The accumulated primary literature on various aspects of this subject now merits this degree of specialization. More general references on yeast include volumes edited by Cook,[3] and by Rose and Harrison.[4] The text by Phaff et al.,[2] includes an introduction to yeast biology.

The cell envelope consists of the plasma membrane, the periplasmic space, cell wall, and (for some species) a slime layer. A schematic representation is given in Figure 1, primarily to establish relative location of components rather than actual dimensions. The cell envelope is thus bounded on the inside by cytoplasm, and on the outside by the medium.

The definitions that follow were developed with the assistance of contributors and other colleagues and have been applied consistently. In the majority of cases I have chosen not to belabor synonymy. Also, inappropriate terms or incorrect applications of terms in current usage have been avoided. One outstanding example of the latter from the older literature is the generic term "membrane" which was sometimes used to describe any membrane of the protoplasm (including the plasma membrane), the cell wall, or even the cell envelope. In the current setting this is, of course, intolerably confusing.

The plasma membrane is the boundary of the cell protoplasm and is microscopically discernible as a bilayer. The plasma membrane is the primary site for solute regulation between the cytoplasm and the medium. Plasma membrane, plasmalemma, ectoplast, protoplasmic membrane, and cytoplasmic membrane are used synonymously in the literature. Semantic arguments against the last two terms have been registered; plasma membrane is in vogue although plasmalemma is frequently encountered. Ectoplast is valid but has not been popularly embraced.

Cytoplasm plus plasma membrane is defined as protoplasm. The protoplasm of a cell gives rise to a protoplast, i.e., an unnaturally produced entity derived from a cell by denuding the cell of cell wall (and slime layer).

Table 1
ABBREVIATIONS FOR YEAST GENERA

Bullera	*B.*	*Nematospora*	*Nem*
Candida	*C.*	*Pichia*	*P.*
Cryptococcus	*Cr.*	*Pityrosporum*	*Pit.*
Debaryomyces	*Deb.*	*Rhodotorula*	*Rh.*
Endomycopsis	*E.*	*Saccharomyces*	*Sacch.*
Hanseniaspora	*H'spora*	*Saccharomycodes*	*S'codes*
Hansenula	*H.*	*Saccharomycopsis*	*S.*
Issatchenkia	*I.*	*Schizosaccharomyces*	*Schiz.*
Kloeckera	*Kl.*	*Sporidiobolus*	*Sporid.*
Kluyveromyces	*K.*	*Sporobolomyces*	*Sp.*
Leucosporidium	*Leu.*	*Sterigmatomyces*	*St.*
Lodderomyces	*Lod.*	*Torulopsis*	*T.*
Lipomyces	*L.*	*Trichosporon*	*Tr.*
Metschnikowia	*M.*	*Trigonopsis*	*Trig.*
Nadsonia	*N.*		

Following Lodder, J., *The Yeasts*, North-Holland, Amsterdam, 1971.

The periplasmic space is a region of the cell envelope which is bounded by the plasma membrane and the inner aspect of the cell wall. This space includes invaginations in the plasma membrane and outward excursions into the inner aspect of the cell wall. The periplasmic space is the locale for several digestive enzymes that act upon substrates that require hydrolysis prior to assimilation by the protoplasm.[5] Physical measurements[6] indicate that reversible changes in the size of the periplasmic space attend the temporary exposure of cells to high osmotic pressure in the bathing medium.

The cell wall is an integument to the cytoplasm and is that part of the cell envelope which confers mechanical stability and dictates shape. For the majority of yeast species the cell wall is the outermost anatomical region. Under normal conditions the yeast cell is turgid and the osmotic potential of the protoplasm is counteracted by wall pressure.

The slime layer is a peripheral region of the envelope which is associated more or less firmly with the outer aspect of the cell wall. An alternate name is "capsule". Cells endowed with capsules are consequently more bulky and tend to be less easily phagocytized by animal cells—a point of some significance about the pathogenic species *Cryptococcus neoformans*. Slime layers may also contribute to characteristic pellicle formation in liquid cultures of some species.

The cell envelope is the interface between the cytoplasm and the medium. Some enzymes and other polymers are secreted by certain yeast species, at least under particular growth conditions. In that context the authors have included some discussion of secreted products from the point of view that these compounds must cross the cell envelope.

One must remark on an antiquated and invalid view that components external to the plasma membrane are "extracellular". Enzymes or other constituents in this category should be referred to as "extraprotoplasmic". The appropriate adjective for components of the cell envelope and secreted items is "extracytoplasmic". Given the current degree of resolution within the yeast cell envelope it is more than semantic to worry about choice of locational prepositions. For example, there are clear distinctions among "on" (which implies a surface location), "within" (which implies an internal locale), and "throughout" (which implies general distribution) when used in conjunction with a particular enzyme and a specific anatomical region of the envelope. I hasten to add that while such distinctions are the goal, the data are not always at hand, and we are often forced to use "associated with" or some other less definitive language.

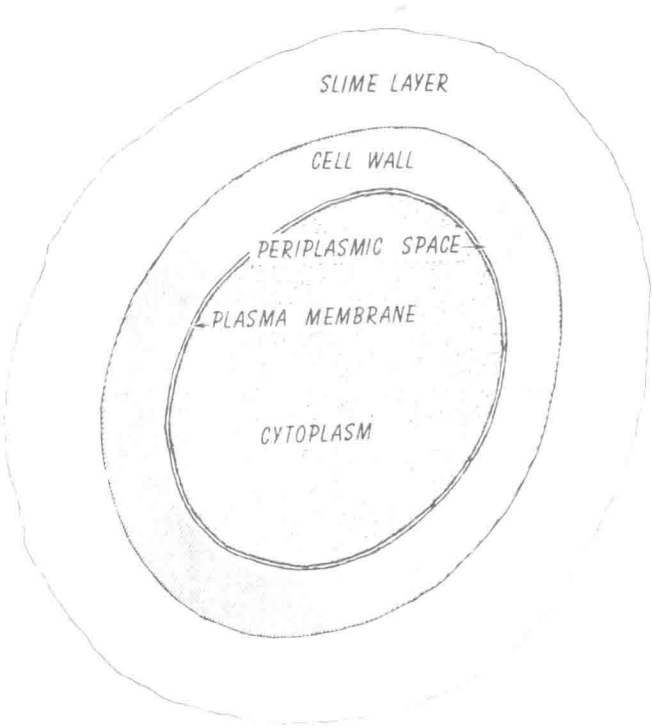

FIGURE 1. Schematic cross section of a yeast cell. The cell envelope is composed of plasma membrane, periplasmic space, cell wall, and slime layer.

The micrographs used herein have magnification bars but a minimum of labels. This is intentional because further appellations might distract more than inform. With respect to graphs, curves were redrawn without individual data points in order to emphasize the important trends in a particular variable. The aim has been to summarize primary data and to provide illustrative micrographs.

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Chapter 2

ATLAS OF CELL MORPHOLOGY

R. G. Garrison and W. N. Arnold

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I. INTRODUCTION

The forms exhibited by yeasts are the consequences of the nature and individuality of their cell envelopes. Some species present a mucilagenous layer as the outermost aspect, but it is the cell wall which contributes most to overall shape. The architecture of the wall is determined by the molecular biology of wall generation (see particularly Chapters 3 and 4 in Volume II). The goals of the present chapter are to document the range of cell shapes encountered in yeasts and to describe the various modes of vegetative reproduction as they reflect on envelope morphology.

The size of all yeast species is within the resolution range of the light microscope and most descriptions have been made at this level. Thus examinations of wet mounts at magnifications of × 400 and × 1000 will continue to be of primary importance for any preliminary morphological examination as well as for routine monitoring of experimental cultures.[1] However, the documentation of yeast morphology by scanning and transmission electron microscopy is now an established reality and this chapter will be devoted to characterization by electron micrographs. The magnifications are × 5000 or more and the detail is commensurately increased.

Cell shape is sometimes useful as a taxonomic criterion; the pyramidal-shaped cells of *Trigonopsis* and the lemon-shaped cells of *Hanseniaspora* are spectacular examples. But for the most part cell shape is a difficult criterion to apply since both size and shape may be influenced by environmental and nutritional factors (see also Volume II, Chapter 8). Moreover, cellular dimensions have limited systematic validity because of ploidy variations.

The great majority of yeast species exhibit budding. A few genera divide by fission, and three genera produce ballistospores as well as buds. In arranging this atlas the authors have employed these three subdivisions. Finally, they present a selection of yeastlike fungi which cause mammalian diseases. For the most part the electron micrographs assembled herein were obtained on specimens grown and processed in the authors' laboratories; those that have been contributed by colleagues are gratefully acknowledged here and are so referenced in the captions. Discussions of fixation and processing of samples are deferred to Volume II, Chapter 8. Our pictorial treatment aims at providing a morphological background for subsequent chapters.

II. MODES OF VEGETATIVE REPRODUCTION

A. Budding

1. Monopolar

In the genus *Pityrosporum,* repeated budding occurs exclusively at one pole. A scar is left on the wall following release of the bud and as successive buds are produced in sequence the scar widens. Cumulative scars form a compound collar from which the younger buds emerge. After separation daughter cells have a birth scar from which subsequent buds appear. This is in contradistinction to the case with multipolar budding in which one morphologically distinct birth scar is carried by the mature cell. Examples of whole cells and thin sections of *Pit. ovale* are shown in Figure 1.

Monopolar budding may be restricted to the genus *Pityrosporum.* A possible exception is *Malassezia furfur,* the etiological agent of the chronic mycotic disease tinea versicolor. Although unsettled there is a contention that *Malassezia* is the invasive form of *Pit. orbiculare.*[2] *M. furfur* has a hyphal stage not yet encountered in *Pityrosporum* species, but otherwise there is a close similarity in the morphology of these yeasts. Electron microscopy of thin sections[2-7] and replicas of freeze-fractured whole cells[8,9] of the *Pityrosporum-Malassezia* group reveal a cell envelope unlike that seen in any other

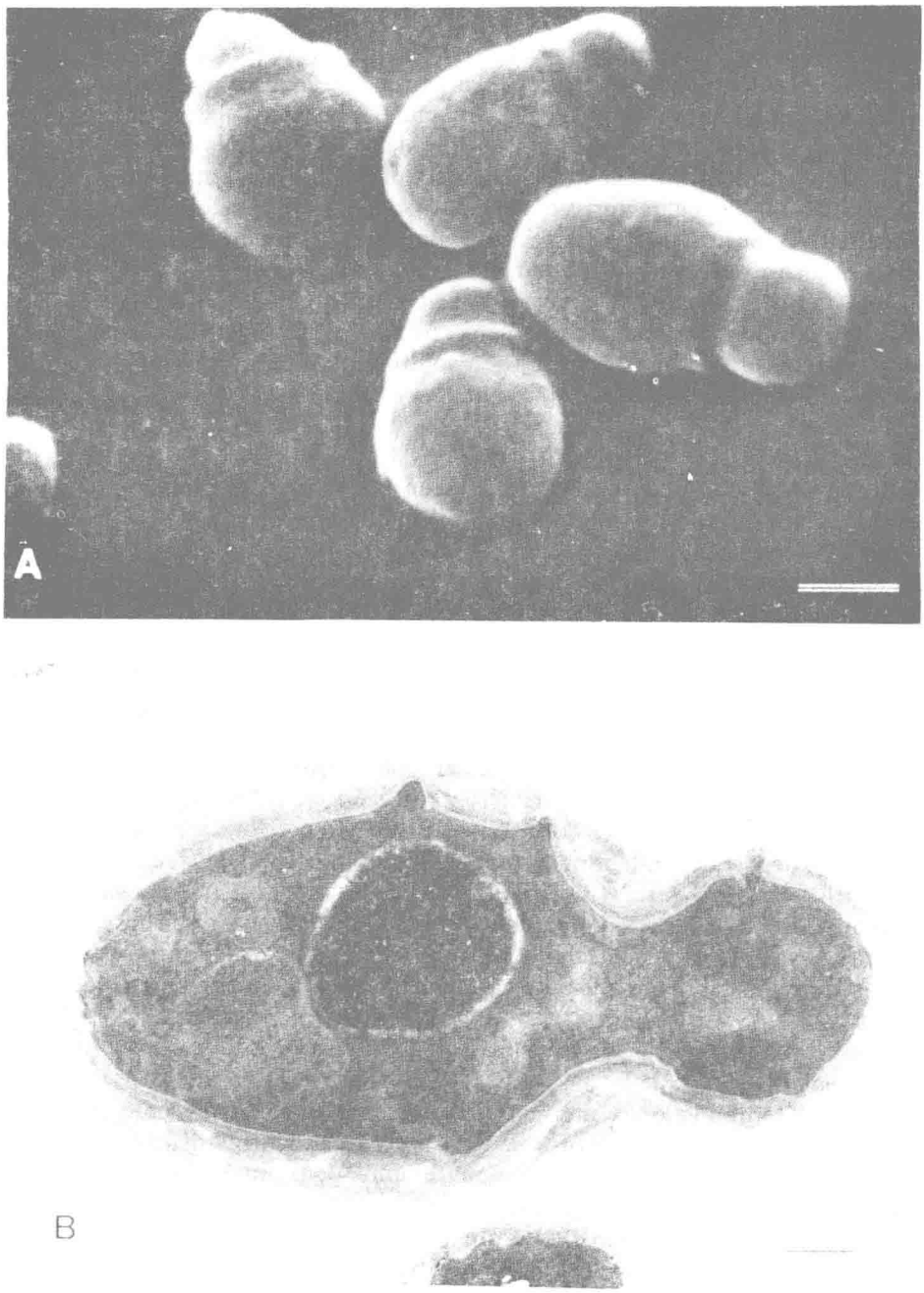

FIGURE 1. Monopolar budding. (A) Scanning electron micrograph of *Pityrosporum ovale*. Bar equals 1.0 μm. (Courtesy of M. N. Miller), (B) thin section of *Pit. ovale*. (Glutaraldehyde-osmium tetroxide.) Bar equals 0.25 μm.

yeast. An account of the unusual nature of this cell envelope is found in Volume II, Chapter 8.

2. Bipolar

Bipolar budding refers to the production of buds at two loci only. The loci are invariably at the poles of elongated cells. This form of vegetative reproduction has been referred to as "budding on a broad base" or "bud fission" and suggests an intermediate mechanism between budding and fission. Bipolar budding leads to the formation of characteristic scars in the cell wall and compound collars at both poles of the cell. Bipolar budding is characteristic of yeasts of the subfamily Nadsonioideae; a representative species, *Hanseniaspora osmophila* is shown in Figure 2. The genus *Kloeckera* also exhibits bipolar budding.

Several authors suggest that yeasts which exhibit bipolar budding may have a common ancestor. The apiculate yeasts may be intermediate between fungi exhibiting true mycelium and budding yeasts; the evolutionary development of the apiculate yeasts could have been in either direction.[10]

3. Multipolar

When buds are formed at different sites on the surface of the parental cell, the budding is referred to as multilateral or multipolar. This mode of vegetative reproduction is by far the most common among the yeasts and many reports on the biochemical and biophysical mechanisms of multipolar budding have been published. The best studied species is *Saccharomyces cerevisiae* (see for example Matile et al.[11]).

A prominent bud scar and a less obvious birth scar result from this type of cell division. The number of bud scars in a mature cell corresponds to the number of daughter cells produced; for example two bud scars and one birth scar are visible in the cell of *Sacch. rouxii* undergoing budding (Figure 3A). Both bud and birth scars can be enhanced with brighteners under fluorescence microscopy (see Volume II, Chapter 4). Figure 4A shows a scanning electron micrograph of *Sacch. cerevisiae* with a multiplicity of bud scars apparently randomly distributed. The number of bud scars observed in thin section is obviously dependent on the plane of section examined (see Figures 3B and 4B). Birth scars are rarely discerned in cross section by transmission electron microscopy. Belin[12] has shown that buds can develop over parts of birth scars whereas bud scars never overlap each other. Thus the topography of the birth scar, which is never as prominent as that of the bud scar, tends to become obliterated in older cells, at least in *Sacchuvarum*.

4. Sterigmatal

Vegetative reproduction by sterigmata is restricted to the genus *Sterigmatomyces*.[13] This genus includes six species of which two were isolated from marine sources; they form neither classical buds nor true mycelium. Vegetative reproduction by the formation of a conidium on a tube-like sterigma is readily discerned in scanning (Figure 5A) and transmission (Figure 5B) electron micrographs. Mature cells may produce one or more sterigmata each giving rise to a single conidium, and after separation of cells protrusions remain (Figure 5A). Whether the septum is equidistant between parent and daughter cells, or closer to the parental cell, seems to differ among species. Kreger-van Rij and Veenhuis[14] studied wall structure and conidium ontogeny in *St. halophilus* by transmission electron microscopy of permanganate-fixed material. They concluded that *Sterigmatomyces* may be related to the basidiomycetous yeasts on the basis of a seemingly laminated wall (see Volume II, Chapter 8, Figure 6B); bud envelopes appeared to have less layers than parental cells. This may be so; however, it seems

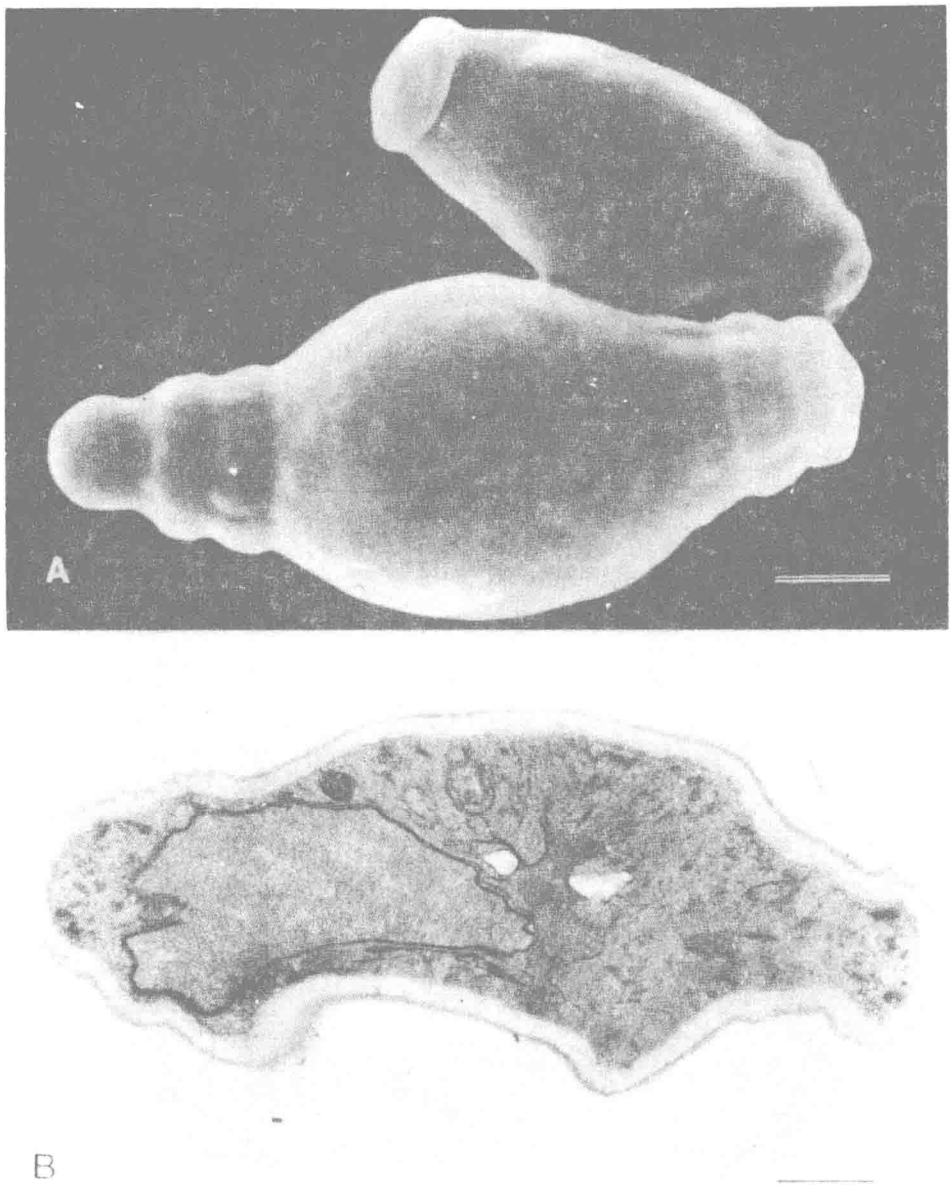

FIGURE 2. Bipolar budding. (A) Scanning electron micrograph of *Hanseniaspora osmophila*. Bar equals 0.5 μm; (B) thin section of *H'spora osmophila*. (Permanganate.) Bar equals 0.5 μm.

possible to us that the outer regions of the envelope are more like slime layers and the relative degree of retention during permanganate fixation may vary with cell envelope age. This in turn gives rise to an apparently denuded bud in *St. halophilus*.[14] This situation is in contrast to that in *Sacch. cerevisiae* in which bud walls never differ much in thickness from those in parental cells.[15] We suggest that the cell wall proper is continuous between conidium and parental cells of *St. halophilus*. The claim that buds