Retinoblastoma-related proteins (RBRs) are key regulators and hubs in a complex regulatory network that controls cell cycle and cell division. RBRs have also been shown to play a role in the regulation of a wide variety of other cellular processes including tumor suppression, DNA repair, DNA damage checkpoint control, differentiation, cellular senescence and apoptosis. The factors directly interacting with RBRs are cyclins, kinases, transcription factors and chromatin remodeling complexes. The retinoblastoma tumor suppressor pathway has been investigated in detail in higher organisms as it was assumed that RBRs only have a widespread role in advanced multicellular development. This review gives an overview of the phylogenetic distribution of RBRs and particularly emphasizes the presence of RBRs in lower eukaryotes. RBRs were not only identified in animals (Metazoa) but also in Viridiplantae (Monocots, Dicots, Lycophytes, mosses and green algae), Heteroconota (Oomycetes, diatoms and brown algae), Alveolata, red algae (Rhodophyta), Haptophyta and Amoebozoa. Only the Fungi seem to be an exception, because only the most primitive phylum retained an RBR, while the more advanced phyla evolved a functionally similar key regulator instead. RBRs are clearly not a particular characteristic of multicellular eukaryotes, as they are also common to unicellular eukaryotes. Thus, RBRs are highly conserved and much more widespread among eukaryotes than previously thought.

Introduction

The foundations for research in the retinoblastoma (RB) tumor suppressor pathway were laid in 1971 when Alfred Knudson proposed that retinoblastoma, a cancer of the eye in children, was initiated by the inactivation of a putative tumor suppressor gene. This hypothesis was subsequently confirmed by demonstrating a loss of heterozygosity at the RB gene locus in retinoblastomas and by cloning the human RB gene (HsRB1, Figs. 1 and 2), which was the first tumor suppressor gene identified. Since these early studies, it has been revealed that the RB protein is a key regulator of the cell division control machinery and that it is lost or inactivated in most human tumors. Moreover, it has become clear that RB-related proteins (RBRs) affect many other biological processes, such as DNA repair, DNA damage checkpoint control, differentiation, cellular senescence and apoptosis. RBRs act as hubs for controlling the cell cycle by connecting the cell cycle clock with transcription machinery. In this way, banks of genes are controlled via the retinoblastoma tumor suppressor pathway. RBR proteins operate by binding and inhibiting transcription factors of the E2F family and by participating in inhibitory chromatin remodeling complexes. RBR activity is regulated, primarily by post-translational phosphorylation, since transcription factors like E2F only bind the hypophosphorylated form of RB. Phosphorylation of RB is directed by cyclin dependent kinases (CDKs) and leads to the release of E2F/DP transcription complexes, which are then able to activate target genes.

Since the identification of the human RB gene, RBRs have been reported in many other species, and a number of previous reviews deal with the sequence, function and phylogenetic analysis of RBRs. Currently, RBRs from ~120 species are known (see below), in which almost two-thirds come from animal species (Metazoa), almost one-third come from Viridiplantae, and a few come from other phyla. However, this is not representative of the actual distribution of RBRs but seems to mainly reflect the phylogenetic allocation of eukaryotic species that have been sequenced to date.

While this review deals with the phylogenetic distribution of RBRs in general, it emphasizes RBRs from lower eukaryotes because this group has received less attention overall. The search for RBRs in single-celled, lower eukaryotes was of special interest, not only because previous research in the RB pathway has dealt mainly with multicellular development but also due to the hypothesized absence of RBRs in unicellular eukaryotes. A phylogenetic analysis of RBRs from lower eukaryotes provides insight into the evolutionary history of RBRs.

Sequence Data Mining and Phylogenetic Analysis of RBRs

A BLAST search with human RB (HsRB1, Figs. 1 and 2) in the DDBJ/EMBL/GenBank databases reveals over one hundred RBR sequences from various species. Other RBRs can be found by BLAST searches in organism-specific sequence databases, which are accessible through the NCBI Entrez Genome Project Database website...
**Figure 1.** Protein sequence alignment and schematic representation of the A-B contact sites of RBRs. RBRs are from animals (Metazoa), Viridiplantae, Heteroconta, Alveolata, red algae (Rhodophyta), Heterolobosea, Haptophyta, Amoebozoa and Fungi. The protein sequence alignment was performed using the MUltiple Sequence Comparison by Log-Expectation program (MUSCLE). Conserved amino acid residues were shaded using GeneDoc 2.6, with similarity groups enabled. White letters on black background: conserved in >65 percent of the sequences at the corresponding position; white letters on dark gray background: conserved in >45 percent of the sequences at the corresponding position; black letters on light gray background: conserved in >25 percent of the sequences at the corresponding position. For clarity, less-related areas were removed and the number of removed amino acid residues is given in parenthesis. Numbering of the α-helices is based on the crystal structure of Hs RBR1. Source organisms: Animals: BmRBR, *Brugia malayi*; CbrBr, *Caenorhabditis briggsae* AF16; CelinRBR (lin-35, R), *Caenorhabditis elegans*; DmRBR, *Drosophila melanogaster*; GgrB1, *Gallus gallus*; HsrB1 (rB1, rB), *Homo sapiens*; HsrB2 (p130), *Homo sapiens*; HsrBL1 (p107), *Homo sapiens*; MmRB1, *Mus musculus*; NvrBr, *Nematostella vectensis*; TarBr, *Trichoplax adhaerens*. Viridiplantae/green algae: CiMat3, *Chlamydomonas incerta*; CrMat3, *Chlamydomonas reinhardtii*; CsrBr1, *Chlorella sp. NC64A*; Cv rBr1, *Chlorella vulgaris* C-169; MpRBR1, *Micromonas pusilla* CCMP1545; MsRBR1, *Micromonas sp. RCC299*. Viridiplantae/Mosses: PprBL1, *Phycomitrella patens*; PprBL2, *Phycomitrella patens*. Viridiplantae/Dicots: AtrBr, *Arabidopsis thaliana*; ChrrBr, *Chenopodium rubrum*; eerBr, *Euphorbia esula*; NbrBr, *Nicotiana benthamiana*; NtrBr, *Nicotiana tabacum*; PsrBr, *Pisum sativum*; PtrBr, *Populus tremula* x *Populus tremuloides*. Viridiplantae/Monocots: CnRBR, *Cocos nucifera*; ZmrBr1, *Zea mays*; ZmrBr2, *Zea mays*; ZmrBr2b, *Zea mays*; SbrBr, *Scutellaria baicalensis*. Viridiplantae/Oomycetes: PhtrBr1, *Phaeodactylum tricornutum* CCAP 1055/1. Alveolata: eurBr, *Eufolliculina uhligi*. red algae (rhodophyta): CmrBr, *Cyanidioschyzon merolae* strain 10D. Heterolobosea/Amoeboflagellates: NgRBR, *Naegleria gruberi*. Haptophyta/Coccolithophores: EhRBR, *Emiliania huxleyi* CCMP1516. Amoebozoa: DdrBr, *Dictyostelium discoideum* AX4; DpRBR, *Dictyostelium purpureum* QSPDI. Fungi/Chytridiomycetes: BdRBR, *Batrochocytium dendrobatidis* JAM81.
Figure 2. Relationships among RBRs from animals (Metazoa), Viridiplantae, Heteroconota, Alveolata, red algae (Rhodophyta), Heterolobosea, Haptophyta, Amoebaoza and Fungi. The unrooted tree was calculated using the neighbor-joining method of PHYLIP. Numbers indicate bootstrap analysis values obtained using 10,000 resampled data sets. RBR-related proteins and source organisms are as follows: Animals: Bm rBr, Brugia malayi; CbrBr, Caenorhabditis briggsae AF16; CelrBr, C. elegans; CiirBr, Ciona intestinalis; DmrBF, Drosophila melanogaster; GgrB1, Gallus gallus; HsrB1 (rB1, rB), Homo sapiens; HsrB2 (p130), Homo sapiens; HsrB1i (p107), Homo sapiens; MnRB1, Mus musculus; NvrBr, Nematostella vectensis; TrBr, Trichoplax adhaerens; Viridiplantae/green algae: CiMat3, Chlamydomonas incerta; CrMat3, Chlamydomonas reinhardtii; TiRBR, Trichoplax adhaerens. Viridiplantae/mosses: Pp rBL1, Physcomitrella patens; PprBL2, Physcomitrella patens; PprBr, Physcomitrella patens. Viridiplantae/Lycopodiophyta: Sm rBr, Selaginella moellendorffii. Viridiplantae/ Monocots: CnRBR, Cocos nucifera; ZmrBr1, Zea mays; ZmrBr2, Zea mays; ZmrBr2b, Zea mays. Viridiplantae/Dicots: At rBr, Arabidopsis thaliana; ChBr, Chenopodium rubrum; EnrBr, Euphorbia esula; NbrBr, Nicotiana benthamiana; NtBr, Nicotiana tabacum; PbrBr, Pisum sativum; PrBr, Populus tremula x P. tremuloides; SbrBr, Scutellaria baicalensis. Heteroconota/Oomycetes: PhrBr, Phytophthora capsici; PhsBr1, Phytophthora sojae; PhrBr, Phytophthora ramorum; AeBr1, Aphanomyces euteiches; AeBr2, Aphanomyces euteiches. Heteroconota/diatoms: TpRBR, Thalassiosira pseudonana CCMP1335; PrBr, Phaeodactylum tricornutum CCAP 1055/1. Alveolata: EuBr, Euglena sp.; ChBr, Chlorella vulgaris. Red algae (Rhodophyta): CmrBr, Cyanidioschyzon merolae strain 10D. Heterolobosea/Amoebafagellates: NgBr, Naegleria gruberi. Haptophyta/Coccolithophores: EhBr, Emiliania huxleyi CCMP1516. Amoebaoza: DdRBR, Dictyostelium discoideum AX4; DpBr, Dictyostelium purpureum QSDPI. Fungi/Chytridiomycetes: BdRBR, Batrachochytrium dendrobatidis JAMBI.
Then, an unrooted tree was computed using the neighbor-joining method.\(^44\) The A-B contact site, a non-conserved spacer region splits the pocket domain into interface forms through the packing of portions of seven helices, the RBR alignment which includes the A-B interface. The A-B contact site is therefore not included in this analysis.

Alignment of protein sequences was performed using MUSCLE.\(^38\) The main sequence similarity among RBRs resides in the pocket domain. This domain mediates interactions with other proteins to exert the biological functions of this family, and, therefore, RBRs are often referred to as ‘pocket proteins’.\(^39-42\) A non-conserved spacer region splits the pocket domain into an A-box portion and a B-box portion.\(^43\) The A-B contact site, also called the A-B interface, is localized at the end of the A-box and at the beginning of the B box. Figure 1 shows a part of the RBR alignment which includes the A-B interface. The A-B interface forms through the packing of portions of seven helices, three from the A box (α8, α9 and α10) and four from the B box (α11, α12, α13 and α14)\(^42\) (Fig. 1). In most RBRs, the A box is ~160 aa in length, and the B box is ~120 aa in length. The spacer region between the A and B boxes is ~100 aa on average. However, the length of this spacer region varies significantly (Fig. 1). The spacer length in the analyzed animal RBRs is ~20–210 aa (mean value: 107 +/- 70). Compared to animal RBRs, there is somewhat less variability in Viridiplantae; i.e., the length of the spacer is ~100–190 aa (mean value: 134 +/- 26). However, within Viridiplantae, both Monocots and Dicots show constant spacer lengths of ~123 aa (mean value: 123 +/- 1). In Ameobozoa, the lengths are ~60–70 aa, in Heteroconota, and in all other investigated groups, the lengths are below 60 aa.

To perform a phylogenetic analysis, the aligned RBR sequences were each trimmed to a length of ~500 aa using BioEdit v7.0.9,\(^43\) by keeping only the well-conserved segments of the alignment. Then, an unrooted tree was computed using the neighbor-joining method.\(^44\) Figure 2 shows the relationships among a selection of RBRs from animals (Metazoa), Viridiplantae, Heteroconota, Alveolata, red algae (Rhodophyta), Heterolobosea, Haptophyta, Ameobozoa and Fungi.

**RBRs in Animals**

Since the identification of RB1 (HsRBL1), two other RBRs, RBL1 (HsRBL1, p107) and RBL2 (HsRBL2, p130), have been detected in man\(^53,55\) (Figs. 1 and 2). Aside from in humans, RBRs have been found in all other sequenced mammalian genomes, including chimpanzee (Pan troglodytes), rat (Rattus norvegicus), dog (Canis familiaris), cattle (Bos taurus), horse (Equus caballus), and mouse\(^46\) (Mus musculus; MmRBl, Figs. 1 and 2). RBRs were also present in the genomes of other vertebrates, like the avian genome of zebra finch (Taeniopygia guttata) and chicken\(^47\) (Gallus gallus; GgRBl, Figs. 1 and 2), amphibian genomes of frog\(^48\) (Xenopus laevis) and newt\(^49\) (Notophthalmus viridescens), and fish genomes of trout\(^50\) (Oncorhynchus mykiss) and zebrafish (Danio rerio). Members of the RBR family have also been found in many invertebrates. There are known RBRs in simple Chordata like Cephalochordata, e.g., lancelet (Branchiostoma floridae), and tunicates (e.g., Ciona intestinalis; CiRBR, Figs. 1 and 2). RBRs have also been identified in Echinoderms like the sea urchin (Strongylocentrotus purpuratus) or Nematodes like Caenorhabditis elegans\(^51\) (CelRBR, Figs. 1 and 2), Caenorhabditis briggsae (CbRBR), and Brugia malayi\(^52\) (BmRBR, Figs. 1 and 2); the latter causes lymphatic filariasis in humans. Within the class Insecta, RBRs have been found so far in the fruit fly Drosophila melanogaster\(^53\) (DmRBF, Figs. 1 and 2), the mosquitoes Aedes aegypti and Culex quinquefasciatus, and in the honey bee (Apis mellifera). RBRs are also present in Chelicerates like the deer tick (Ixodes scapularis) and Cnidarians like the sea anemone Nemastella vectensis\(^54\) (NvRBR, Figs. 1 and 2), which shows a very simple body plan, as it has only a single orifice for ingestion and excretion and it lacks a head or brain. Even the simplest known animal with the smallest known animal genome, the Placozoan species Trichoplax adhaerens, has an RBR\(^55\) (TaRBR, Figs. 1 and 2). Trichoplax are very flat, disc-shaped creatures, 1–2 millimeters in width that use cilia for locomotion. They have no organs or internal structure and do not even have body axes. Trichoplax consists of two epithelial layers that sandwich a layer of multinucleate fiber cells. They only have four types of body cells, not including nerve, sensory or muscle cells.

The fact that RBRs have not only been identified in the simplest known animals, but also in all other investigated animals, suggests that all animals have RBRs.

**RBRs in Viridiplantae**

RBRs have been identified in several higher plants,\(^31,35,40\) despite the fact that key molecular mechanisms of the RB pathway are conserved in higher plants, some details of the molecular interactions and responses are plant-specific.\(^36,37\) Among the seed-bearing plants (Spermatophyta) RBRs have been identified in Gymnosperms, e.g., pine and spruce\(^38\) and in both monocot and dicot Angiosperms.

Within the Monocots, RBRs have been found in grass species, such as maize (Zea mays), rice (Oryza sativa), wheat (Triticum aestivum), barley (Hordeum vulgare), Sorghum bicolor and sugarcane (Saccharum sp.), as well as in non-grass species such as palm (Cocos nucifera; CnRBR, Figs. 1 and 2) and onion (Allium cepa).\(^31,39,58\) Among the higher plants only grasses, like maize, have more than one RBR in its genome\(^59-62\) (ZmRBRs, Figs. 1 and 2), suggesting recent gene duplication events in this group.\(^58\)
RBRs have also been identified in quite a few Dicots,\textsuperscript{5,19,38} including Arabidopsis thaliana\textsuperscript{53} (AtRBR, Figs. 1 and 2), tobacco\textsuperscript{64} (Nicotiana tabacum; NtRBR, Figs. 1 and 2), Nicotiana benthamiana (NbRBR, Figs. 1 and 2), pea\textsuperscript{60} (Pisum sativum; PsRBR, Figs. 1 and 2), soybean (Glycine max), tomato (Solanum lycopersicum), cotton (Gossypium sp.), grape (Vitis sp.), poplar (Populus tremula x Populus tremuloides; PrRBR, Figs. 1 and 2), leafy spurge (Euphorbia esula; EcRBR, Figs. 1 and 2), red goosefoot (Chenopodium rubrum; ChrRBR, Figs. 1 and 2), Medicago truncatula, Lotus japonicus and Scutellaria baicalensis (SbRBR, Figs. 1 and 2).

RBRs are also present in the most primitive vascular land plants, the Lycophytes (Lycopsida, Primaurophyta), which include the spike moss Selaginella moellendorffii (SmRBR, Figs. 1 and 2). S. moellendorffii has a genome size of only ~100 Mbp, which is the smallest genome size of any land plant reported. Nevertheless, the genome of S. moellendorffii even contains four RBRs.

Within the Bryophytes (mosses), the oldest land plants on earth, Physcomitrella patens is the best studied species. This non-vascular embryophyte has at least three RBR genes in its genome (PpRBL1, PpRBL2, PpRBR, Figs. 1 and 2).

The Viridiplantae subgroups described above contain only multicellular species, whereas the next subgroup, the green algae, includes both multicellular and unicellular organisms. In volvocine green algae, RBRs have been identified not only in the multicellular species Volvox carteri\textsuperscript{26,67} (VcRBR1, Figs. 1 and 2) but also in the unicellular organisms Chlamydomonas reinhardtii\textsuperscript{28,69} (CrMAT3, Figs. 1 and 2) and Chlamydomonas inserta (GiMAT3, Figs. 1 and 2). Within the Trebouxiophyceae, RBRs were found in the unicellular genus Chlorella (Chlorella vulgaris, CvRBR1; Chlorella sp., CsRBR1, Figs. 1 and 2). RBRs are also present in Prasinophyceae, including the genera Micromonas (Micromonas pusilla, MpRBR1; Micromonas sp., MsRBR1, Figs. 1 and 2) and Ostreococcus\textsuperscript{70,71} (Ostreococcus tauri, OtRBR, OtRBR2; Ostreococcus lucimarinus, OIrRBR, Figs. 1 and 2). These are very small, unicellular eukaryotes with low cellular complexity and small genomes. The nucleolar genome of Ostreococcus tauri is only 12.6 Mbp in size, and its cell diameter is less than 1 μm, which makes it the smallest known free-living eukaryote.\textsuperscript{71,72}

Because all previously investigated Viridiplantae have an RBR, whether they are multicellular or unicellular, RBRs are most probably present in all Viridiplantae.

**RBRs in Heteroconta**

The Heteroconta (Stramenopiles) represent another major eukaryotic group and include the Oomyces (water molds), the diatoms (Bacillariophyta) and the brown algae (Phaeophyta). Within the Oomyces, a diverse group of eukaryotic microbes that include saprophytes and pathogens of plants, animals and microbes, so far RBRs have been identified only in the plant pathogenic genera Phytophthora (Phytophthora capsici, PcRBR, Phytophthora sojae, PhsRBR1, Figs. 1 and 2; Phytophthora ramorum, PrRBR, Fig. 2) and Aphanomyces (Aphanomyces euteiches, AeRBR1, AeRBR2, Figs. 1 and 2). In diatoms, the dominant organisms of phytoplankton, RBRs can be found in the centric Thalassiosira pseudonana (TpRBR, Figs. 1 and 2) and the pennate Phaeodactylum tricornutum (PhtRBR1, Figs. 1 and 2). There are no reports of RBRs in brown algae; however, this is probably only the result of a lack of genome sequence information from this group.

**RBRs in Heterolobosea, Haptophyta, Alveolata, Red Algae and Amoebozoa**

Amoeboflagellates (Heterolobosea) are a basal lineage of amoebae. An RBR was identified in the unicellular amoeboflagellate Naegleria gruberi (NgRBR, Figs. 1 and 2), which can exist as an amoeba, flagellate and cyst. Another single-celled organism with an unambiguous RBR gene is Emiliania huxleyi (EhRBR, Figs. 1 and 2), a globally distributed coccolithophore (Haptophyta) covered with uniquely ornamented calcite disks. Within the Alveolata, a group of primarily single-celled eukaryotes that is united by the presence of small vesicles (alveoli) in, or just under, the plasma membrane, RBRs were identified in the unicellular marine ciliate Eutigulculus ubilis\textsuperscript{73} (EuRBR, Figs. 1 and 2) and in the single-celled freshwater ciliate Tetrahymena thermophila. An RBR is also present in the ultrasmall, unicellular red alga Cyanidoschyzon merolae (CmRBR, Figs. 1 and 2), which lives in acidic hot water even at a pH <2. However, most species within the red algae (Rhodophyta), one of the oldest groups of eukaryotic algae, are not single-celled but rather multicellular, macroscopic organisms. Probably due to a lack of genome projects for multicellular red algae, the most primitive unicellular red algae have the only known RBR from Rhodophyta. RBRs have also been identified in another primitive eukaryotic group: the Amoebozoa. These amoeboid protozoa have pseudopodia and move by means of internal cytoplasmic flow. Within the Amoebozoa, RBRs have been found in the true slime mold Phyvarum polycephalum\textsuperscript{8} and in the cellular slime molds of the genus Dictyostelium (Dictyostelium discoideum, DdRBR; Dictyostelium purpureum, DpRBR, Figs. 1 and 2). In cellular slime molds, individual amoebae exhibit the characteristic behavior of forming multicellular organisms under certain growth conditions.

**RBRs in Fungi**

While all groups of eukaryotes mentioned above appear to have RBRs, the situation in Fungi (Eumycota) seems to be different. There is partial or complete sequence information from over one hundred fungal genomes in different databases; however, only a single, clear RBR can be found within these sequences. This RBR comes from the non-hyphal zoosporic fungus Batrachochytrium dendrobatidis (BdRBR, Figs. 1 and 2), which infects the keratinized skin of amphibians and causes the disease chytridiomycosis. B. dendrobatidis belongs to the Chytridiomycota, the most basal fungal phylum. This suggests that RBRs were ancestrally present. However, among recent fungi only the most basal phylum retained an RBR, while more advanced phyla lost the RBR and promoted a functionally similar key regulator of cell cycle entry. Studies in yeast (Saccharomyces cerevisiae) revealed that there is only the remnant of an RB gene (YLR419w) with weak
similarity and without RB function,\textsuperscript{37-39} whereas the negative regulator Whi5 appears to be the functional substitute of RB in these fungi.\textsuperscript{76-80}

### RBRs in Non-Eukaryotes

The stringent BLAST search mentioned above did not reveal any significant RB-related sequences from non-eukaryotes. However, two short amino acid sequences, in the N-terminal and pocket A box regions, were found in two archaeotes (\textit{Archaeoglobus fulgidus} and \textit{Methanococcus jannaschii}), and another short amino acid sequence from the B box region was found in poxviruses.\textsuperscript{37} The caveat to these findings is that regions of similarity are only ten amino acids or less in length, which might not be significant, and the identified sequences only have one of the similar patches, but not both. Therefore, the presence of functional RBRs appears to be restricted to eukaryotes. The evolutionary pathway leading to RBRs in eukaryotes seems have been obscured by the passage of time.

### Conclusions

Not long ago, it was thought that the RBRs were specific to vertebrates,\textsuperscript{11} and somewhat later, after the identification of RBRs in plants, it was speculated that RBRs only have a widespread role in multicellular development,\textsuperscript{35,37,41-82} which requires a complex organization of many cooperating cells and many different cell types to develop complex body plans. However, based on the phylogenetic distribution of RBRs, this hypothesis is no longer tenable. RBRs are more highly conserved in eukaryotes than initially thought. This suggests that the RB pathway may have a more general role in the cellular division of all eukaryotes. The only exception is within the Fungi, which promoted a functionally similar key regulator and secondarily lost the RBR. RBRs are present not only in very simple, lower multicellular eukaryotes but also in unicellular eukaryotes. The presence of RBRs in unicellular eukaryotes is not restricted to one or a few species but instead seems to be the rule. In contrast, there are no RBRs in non-eukaryotes, which might be linked to the function and cellular localization of RBRs: RBRs are found in the nucleus, and their subnuclear localization depends on binding to the nuclear matrix.\textsuperscript{35,83-85} Importantly, some mechanisms of control of the cell cycle correlate with the compartmentalization of RBR within the nucleus.\textsuperscript{35,85} Given that the nucleus is essential for the key function of RBRs, the presence of a nucleus in eukaryotes and its absence in non-eukaryotes, i.e., Archaea and Bacteria, might be a major reason for the described phylogenetic distribution of RBRs.

Research on RBR function has resulted in tremendous progress over the last two decades; however, this research was strictly focused on higher eukaryotes. The fact that RBRs are much more widespread among eukaryotes than previously thought might provide an incentive for future studies on the basic characteristics of RBRs in eukaryotes with low organismal complexity.

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