in O_2 affinity without large changes in structure (*18*).

It is clear that thermodynamically/energetically significant waters of solvation are not equivalent to the much more tightly bound waters that can be seen by x-ray or neutron diffraction although these "visible" waters can be among those seen thermodynamically.

Intermediate states have been recently postulated for the R-T transition in Hb (*19*). Can the total 60 water molecules we measure be assigned to specific molecular events and the osmotic stress method able to recognize structural intermediates?

The action of other ligands must be reexamined to recognize their concomitant influence on water activity. Here we have only considered the two extreme cases: that there is only direct solute binding ($\Delta n_w = 0$) or only an indirect effect of solute on water binding ($\Delta n_X = 0$). Considering only water binding gives the best agreement between thermodynamic expectations and the experimental data for these solutes. A more general treatment for solutes that do bind as well as change water properties would include both direct and indirect actions. We will present elsewhere work reanalyzing the effect of Cl^- on Hb in terms of both water and ion binding (*20*).

Solvent influence on regulation of Hb-based artificial blood preparations can differ from that of the highly controlled intracellular milieu of erythrocytes. Water activity must be a consideration in system design.

Finally, one can see a unity between allosteric proteins and the several transport proteins (*5*, *6*) where different functional states have measurably different levels of hydration.

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21. Human red cells were washed three times in isotonic salt solutions and lysed with distilled water. Debris was removed by 15,000-rpm centrifugation at 4°C for 1 hour with a Sorvall RC-5B Centrifuge. Protein solutions were eluted over a PD10 Sephadex G-25M column (Pharmacia) in 0.1 M NaCl, 0.05 M tris, pH 7.5, and Hb was concentrated by centrifugation with a Centricon-30 microconcentrator (Amicon). All pH measurements were done with a Radiometer pH meter 26. The preparation procedure was slightly modified from that described by A. Riggs [*Methods Enzymol.* **76**, 5 (1981)].

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An Origin of DNA Replication and a Transcription Silencer Require a Common Element

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A eukaryotic chromosomal origin of replication was identified in the yeast *Saccharomyces cerevisiae*. By several criteria, including map position, deletion analysis, and a synthetic form of saturation mutagenesis, the origin co-localized with the *HMR-E* silencer, which is a DNA element that represses transcription of the adjacent genes. A specific site within the silencer was required for both initiation of chromosomal replication and for repression of transcription. This analysis directly demonstrates that initiation of eukaryotic chromosomal replication is dependent on specific sequence elements and that a particular element can act in both initiation of chromosomal replication and regulation of transcription.

DNA replication is perhaps the most precisely regulated protein–nucleic acid interaction in biology; each base pair in the genome is replicated once and only once every cell division. Much of this regulation of replication may occur at chromosomal origins, the sites at which replication initiates. Specifically, in eukaryotes many origins must initiate to replicate the entire genome yet reinitiation at all origins must be prevented until the next cell cycle. Eukaryotic initiation of replication is regulated in at least two additional ways. Initiation is regulated during development of multicellular organisms; fewer origins initiate late in development than early in development. Initiation is also regulated temporally during S phase; different chromosomal origins or clusters of origins initiate replication at distinct times during S phase (*1*, *2*). Little is known about the molecular mechanisms that control initiation of eukaryotic DNA replication largely because chromosomal origins are ill-defined. Although a few chromosomal origins have been mapped to within a few hundred to a few thousand base pairs, no specific sequence element has been shown to be required for the initiation of chromosomal replication (*3*–*5*).

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We have focused on two aspects of DNA replication; namely, the identification of sequences required for chromosomal initiation and the possible relation between DNA replication and repression of transcription. Two lines of evidence demonstrate that DNA replication and transcription can share regulatory mechanisms. First, initiation of viral replication often depends on sequence elements and proteins that also activate transcription (*6*). Thus, eukaryotic DNA replication and transcription apparently can share common activation mechanisms. Second, activation of transcription of the late genes in bacteriophage T4 and adenovirus is dependent on replication of the phage or virus. Thus, the process of replication itself can activate transcription (*7*).

The mating-type genes of *S. cerevisiae* provide a context in which to study the possible relation between DNA replication and repression of transcription. The mating-type genes reside at three loci, *MAT*, *HML*, and *HMR*. At *MAT* (the mating-type locus) the genes are transcribed and, as a result, govern cell type. Cells carrying the *MAT***a** allele have the **a** phenotype and cells carrying the *MAT*α allele have the α phenotype (*8*). In contrast to the genes at *MAT*, the mating-type genes at *HML* and *HMR* are repressed and do not contribute to

control of cell type. The mating-type genes at *HML* and *HMR* are identical to the alleles at *MAT*. The repressed state of these genes depends on their location at *HML* or *HMR* and on the genetic properties of the *HML* and *HMR* loci (*9, 10*). The repressed state is heritable; once the repressed state is formed it can be propagated through many rounds of cell division (*11*). Repression at *HML* and *HMR* is believed to be mediated by a particular chromatin structure and requires the action of a number of gene products, including the four *SIR* proteins (*12*).

Circumstantial evidence suggests that DNA replication may play a role in repression of transcription of the silent mating-type genes. In particular, if *HMR* is experimentally derepressed in vivo, it remains derepressed until the cells pass through S phase. Thus, some S phase event, perhaps DNA replication, is required for the formation of the repressed state (*13*). Furthermore, the flanking DNA elements that are required for repression at *HML* and *HMR*, known as silencers, may play a role in DNA replication. Each of these silencers, when contained on a plasmid, allows autonomous replication of that plasmid (*14–16*). However, identification of a genomic sequence that confers autonomous replication to a plasmid does not imply that the sequence functions to initiate chromosomal replication. In fact, only a fraction of yeast sequences that allow autonomous replication of plasmids map to chromosomal origins of replication (*17*).

To determine directly whether the *HMR-E* silencer is a chromosomal origin of replication, we used a technique for mapping of replication origins (*4, 18*). This technique relies on the ability of two-dimensional electrophoresis and DNA blot hybridization for distinguishing different forms of in vivo replication intermediates for a given section of the genome. Two forms of replication intermediates, fork-shaped and bubble-shaped, are relevant to this analysis. If a particular genome-derived restriction fragment does not contain an origin of replication, the replication intermediates of this fragment will be fork-shaped because a replication fork will proceed through the fragment from one end to the other. If, however, the fragment contains an origin of replication, the replication intermediates will be bubble-shaped because two forks will proceed bidirectionally from the origin. These shape differences are distinguishable after two-dimensional agarose gel electrophoresis: fork-shaped replication intermediates give rise to one type of arc pattern, whereas bubble-shaped intermediates give rise to a second type of arc pattern (Fig. 1) (*4, 18*). Thus, fragments containing an origin of replication can be unambiguously identified.

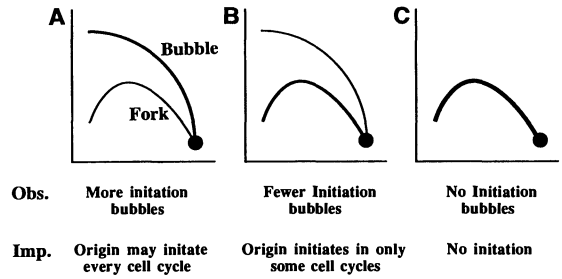

Fig. 1. Analysis of DNA replication intermediates generated in vivo. Upon two-dimensional electrophoresis and DNA blot-hybridization, DNA replication intermediates give rise to a number of distinct arc patterns (*4, 18*). Only two patterns are relevant to the work presented here. Bubble-shaped intermediates give rise to the upper arc (labeled Bubble), whereas fork-shaped intermediates give rise to the lower arc (labeled Fork). The position of non-replicating DNA is indicated as a black circle. Different origins can initiate replication with different efficiencies (*2–4*). The efficiency of initiation is reflected in the ratio of bubble-shaped intermediates to fork-shaped intermediates. This ratio allows relative distinction among origins that (**A**) initiate more frequently, (**B**) less frequently, or (**C**) not at all. The ratio is not a quantitative indicator of the absolute efficiency of initiation (*2, 19*). Obs., observed; Imp., implied.

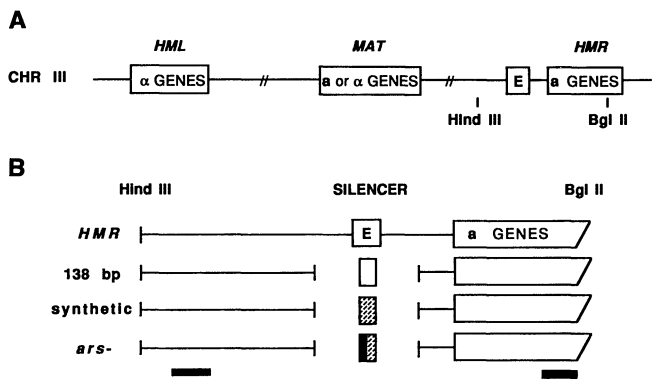

Fig. 2. Schematic representation of the mating-type genes. (**A**) The three copies of the mating-type genes are located on chromosome III (CHR III). The position of the *HMR-E* silencer is labeled E. (**B**) An expanded representation of the Hind III–Bgl II region of *HMR* diagramed in (A). A 0.8-kb region containing the *HMR-E* silencer and origin was replaced with a 138-bp sequence identical to the wild-type sequence (open box), a synthetic silencer (shaded box), or the synthetic silencer with an 8-bp substitution mutation in the ARS consensus sequence (shaded box with black section) (*16*). The relative positions of the probes used are indicated by the thick, horizontal black lines.

The replication intermediates of a restriction fragment containing the *HMR-E* silencer (Fig. 2) were isolated and examined by the two-dimensional origin mapping technique. Both fork-shaped and bubble-shaped intermediates were detected (Fig. 3A). The presence of bubble-shaped replication intermediates unambiguously demonstrates that an origin of DNA replication is located at or near the *HMR-E* silencer. Fork-shaped molecules either could arise from the breakage of bubbles during DNA isolation (*19*) or could represent actual replication intermediates, indicating that this origin does not initiate replication every cell cycle (*2, 4*).

The sequences required for initiation of replication were precisely localized by an analysis of deletion mutants. An 0.8-kb chromosomal region was identified that contained both the *HMR-E* silencer and the origin of replication (*20*) (see below). Smaller fragments were inserted into the chromosome in place of this fragment to test for their ability to direct initiation of replication (Fig. 2). The minimum silencer fragment previously shown to contain all the *HMR-E* sequences required for repression of transcription is a 138-bp fragment (*14, 16*). This fragment contains the three elements that regulate repression: two transcription factor "binding" sites, one each for the RAP1 and ABF1 proteins, and a match to the *ARS* consensus sequence (autonomously replicating sequence) (*20, 21*). A match to the *ARS* consensus sequence is found in all elements that allow autonomous replication of yeast plasmids (*22, 23*). This minimal silencer fragment was integrated into the chromosome in place of the 0.8-kb region that contained the wild-type silencer and origin. To determine whether the origin of replication co-localized with this minimal silencer, the corresponding chromosomal replication intermediates were isolated and then analyzed by the two-dimensional origin mapping technique. The detection of bubble-shaped intermediates demonstrates that this fragment of DNA supports initiation of replication (Fig. 3B). Therefore, the chromosomal origin localized to the minimum wild-type *HMR-E* silencer.

To determine whether the silencer itself was the origin of replication, we examined the replication properties of a synthetically constructed DNA element. This synthetic construct was designed to identify the func-

tional elements within the 138-bp minimal silencer by a strategy akin to saturation mutagenesis. Each of the three elements that contribute to silencing was mutated so that the sequence was changed but the presumed function was preserved. Specifically, the *ARS* sequence at *HMR* was replaced with another version of the *ARS* consensus sequence. Similar substitutions of the transcription factor binding sites allowed mutation of these elements without disruption of their presumptive function. In particular, the ABF1 binding site was replaced by a more symmetric derivative that binds the ABF1 protein more tightly in vitro. Likewise, the RAP1 binding site of the synthetic silencer was identical to a RAP1 binding site from a transcriptional promoter region (*24*). The correct spacing among the known silencer elements and the overall base composition were preserved. However, the sequence identity of the intervening DNA was drastically altered. In total, 71 out of 138 bp were altered between the synthetic silencer and wild-type silencer. Thus, any cryptic site that might lie between the known silencer elements would be heavily mutagenized. This synthetic element repressed transcription at *HMR* when inserted into the chromosome in place of the 0.8-kb region (*16*). To determine if this saturation mutagenesis had impaired the origin, we isolated and analyzed the appropriate chromosomal replication intermediates. The detection of bubble-shaped intermediates demonstrated that this synthetic element acts as a chromosomal origin of replication (Fig. 3C). Thus, the functional elements that comprise the origin of replication co-localized with the functional elements that comprise the *HMR-E* silencer.

The repressed state at *HMR* affects both transcription and other physiologically important protein–nucleic acid interactions over a region of DNA. For instance, HO endonuclease cleavage and at least one form of DNA repair are blocked at *HMR* but not at *MAT*. These effects are alleviated in *sir* strains and thus are likely to be caused by the same mechanism that represses transcription (*9*, *25*). To determine whether the *SIR*-dependent state of *HMR* affected initiation of replication from the *HMR-E* silencer, we isolated and analyzed replication intermediates from an isogenic set of strains that differed only by mutations in the *SIR* genes. Each mutant strain contained a null *sir* allele that resulted in derepression of the silent mating-type loci (*15*). Loss of *SIR1*, *SIR2*, *SIR3*, or *SIR4* function did not prevent chromosomal initiation at *HMR-E* (Fig. 4) (*26*). These results demonstrated that the transcriptionally repressed state of the *HMR* locus was not required for initiation of replication from *HMR-E* and that the individual SIR proteins were not required components of the initiation machinery at the *HMR-E* origin.

The requirement of the *ARS* consensus for initiation of replication was assayed to ascertain the correspondence between silencing function and origin function. The properties of the *ARS* consensus sequence, when contained on a plasmid, make it a strong candidate for an element that is involved in DNA replication, and a substitution mutation in the *ARS* consensus sequence of the synthetic silencer results in derepression of *HMR* (*2*, *16*). This mutation resulted in complete loss of bubble-shaped replication intermediates (Fig. 3D). This result demonstrates that (i) the *ARS* consensus sequence is required, at least in our experiments, for the initiation of chromosomal replication and (ii) a single element is required for initiation of chromosomal DNA replication and for repression of transcription at *HMR*.

Our data allow several conclusions regarding chromosomal origins. No specific sequence element has been shown to be required for initiation of replication in the chromosome, although a wealth of data suggests that eukaryotic replication is directed by specific DNA sequences (*2*, *6*). Demonstration that the *ARS* consensus element is required for initiation is direct evidence that chromosomal initiation of replication is dependent on specific sequence elements.

These data also suggest that the *HMR-E* origin consists of (i) sequences that are required for initiation and (ii) sequences that contribute to the efficiency of initiation. The frequency of initiation from an origin is qualitatively reflected in the ratio of the signal strength of the bubble arc to that of the fork arc (Fig. 1) (*27*). The ratio of bubble-shaped intermediates to fork-shaped intermediates is lower for the 138 bp and synthetic silencers than for the wild-type silencer (Fig. 3). Therefore, these reconstructed silencers initiate replication in fewer cell cycles than the wild-type silencer. A likely explanation for the decrease in initiation of these reconstructed silencers is that the reconstructed silencers lack sequences that contribute to the efficiency of initiation in the wild-type silencer. In this regard, the DNA that is present in the wild-type silencer and is missing in the reconstructed silencers is AT-rich and contains a number of near (9 out of 11 bp) matches to the *ARS* consensus sequence (*15*). These AT-rich sequences may contribute to the efficiency of initiation in a number of ways. For example, by analogy to transcription enhancer elements, these se-

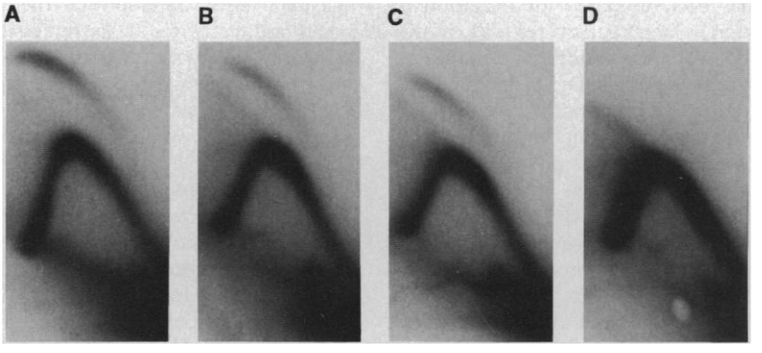

Fig. 3. Comparison of chromosomal replication intermediates derived from different versions of the *HMR-E* silencer. (**A**) Wild-type silencer (yeast strain DBY703). (**B**) The 138-bp segment of the wild-type silencer (JRY2148). (**C**) Synthetically constructed silencer (JRY2879). (**D**) Synthetic silencer with a mutant *ARS* consensus sequence (JRY2881). Yeast strains were described previously (*16*). Analysis of replication intermediates was as described (*18*) with the following exceptions. One-liter cultures (0.95 to 1.4 A_{600}) were harvested (*34*). Glycerol (to 20%) was added to the 0.2 M EDTA solution to facilitate rapid thawing. After thawing, cells were washed in cold water, resuspended in 1X NIB (*35*) and stored at −70°C. DNA (100 mg), prepared as described (*35*), was digested with the appropriate enzyme (or enzymes) for 2 hours at 37°C. Replication intermediates were then enriched by BND-cellulose chromatography (*36*). Two-dimensional electrophoresis was carried out as described (*18*). The DNA was transferred to Zeta-probe membrane as described by the manufacturer (Bio-Rad). Probe labeling (Multiprime) and hybridization were as recommended (Amersham), except that two probes were often used to increase sensitivity (*37*). For this particular experiment, Hind III and Bgl II restriction enzymes were used (Fig. 2). This digestion resulted in an approximately 3.8-kb wild-type genomic fragment in which the silencer was centrally located (2.2 kb from one end). The reconstructed silencers were also centrally located (1.7 kb from one end) but were on smaller (3.2 kb) fragments. In addition to the fork signal in (D), a second signal is detectable emanating from the top of the fork arc. This arc pattern is expected for double fork-shaped replication intermediates that are formed when two forks meet and terminate replication (*4*). The detection of this form of intermediate does not alter the conclusions presented.

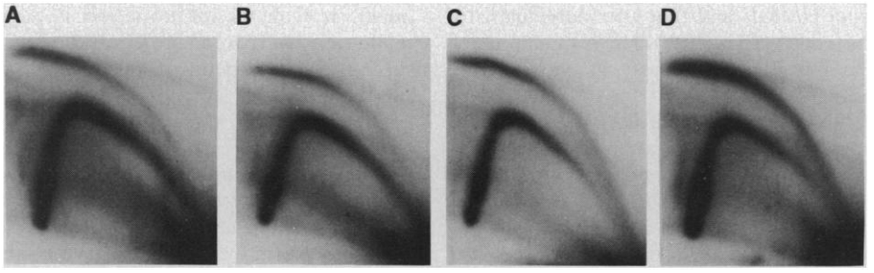

Fig. 4. Analysis of *HMR-E* replication intermediates in isogenic *sir* strains. (**A**) Wild-type control (DBY703), (**B**) *sir1* (YWK178), (**C**) *sir2* (YWK179), (**D**) *sir3* (YWK55). These strains were described (*15*). Replication intermediates were analyzed as in the legend to Fig. 2. In this particular experiment, the Hind III restriction enzyme was used to generate a 5-kb genomic *HMR-E* fragment in which the silencer was 2.2 kb from one end.

quences could bind specific proteins that somehow increase the frequency with which the silencer is used as an origin. Alternatively, the 9 out of 11 bp near matches may act to form a DNA unwinding element, which is an easily unwound region of DNA that has been proposed to contribute to efficiency of initiation (*28*). A third possible explanation for the lower efficiency of initiation in the reconstructed silencers is that the deleted sequences could contain a second origin of replication. A fourth possibility is that the silencer acts to bind a protein complex required for initiation of replication but that the actual initiation event can occur at many sites in the vicinity of the silencer. Such a "zone of replication" has been proposed for initiation of replication in larger eukaryotes (*29*). In this case, the efficiency of initiation would be proportional to the number of sites at which replication could potentially initiate.

The association of silencer function with the *ARS* consensus element does not appear to be a universal feature of chromosomal origins. Although the ARS consensus element of the *HMR-E* origin was required for repression of transcription at *HMR*, matches to the *ARS* consensus are found near a number of yeast genes that are actively transcribed (*23*). Therefore, the *ARS* consensus does not always act to repress transcription of adjacent genes. Similarly, other chromosomal origins of replication are located near actively transcribed genes. For instance, a chromosomal origin of replication maps very near the *TRP1* gene (*2*), and origins are interspersed among the tandemly repeated ribosomal genes (*3, 4*). Therefore, active chromosomal origins do not necessarily repress transcription of nearby genes. Consequently, silencer activity is inferred to be a specialization of the *HMR-E* origin.

Various relations between replication and repression of transcription at *HMR* are possible. At one extreme, replication and transcription functions could be completely unrelated if the silencer bound one set of proteins that initiates replication at one time in the cell cycle and bound another set of proteins that represses transcription at other times. At the other extreme, replication and repression could be mechanistically coupled. The results presented above demonstrated that repression of transcription was not required for initiation of replication from the *HMR-E* origin. However, repression of transcription may, in fact, be a consequence of initiation of replication at the *HMR-E* origin. Replication may be directly required for repression. For instance, the replication machinery assembled at the *HMR-E* origin might include factors required for the formation of a particular chromatin structure. Alternatively, the replication function of the silencer could indirectly result in repression. For instance, eukaryotic origins of replication are proposed to cluster in large structures (*30*). Inclusion of the *HMR-E* silencer in such a structure might preclude transcription from genes in the vicinity of *HMR-E*.

All models proposing that replication is required for repression of transcription are constrained by the frequency of initiation at *HMR-E*. The reconstructed silencers initiate replication in a fraction of cell cycles, yet transcription at *HMR* is repressed in all cell cycles (*16*). How could periodic initiation play a mechanistic role in the continuous repression of *HMR*? The stability of the *SIR*-dependent repressed state is achieved by the combination of a mechanism for establishing the repressed state with a mechanism for inheriting the repressed state (*11, 31*). Recent studies have shown that the establishment and inheritance functions are experimentally separable. The inheritance mechanism can act to maintain the repressed state for at least ten rounds of cell division (*11*). Since the repressed state is stably maintained and efficiently inherited, the repressed state needs only be established in a fraction of the cell cycles to result in repression in all cells. Thus, it is possible that infrequent initiation of replication from the *HMR-E* silencer is required for the establishment of repression of transcription at the *HMR* locus. If the role of the *HMR-E* silencer is to act as an origin of replication, the other silencers should also be origins of replication. However, previous efforts have failed to detect initiation at the *HML* silencers (*17*), possibly because (i) some silencers may not require initiation of replication for repression of transcription and thus may operate by a completely different mechanism or (ii) initiation of replication from the silencers might only be required for the establishment of repression. In that the repressed state can be inherited very efficiently at *HML*, it needs to be established only rarely. As pointed out, the sensitivity of detection of initiation at *HML* was not sufficient to address whether silencer-dependent initiation of replication plays a role in the establishment of repression at *HML* (*17*).

The repression of genes by silencers in yeast is an example of the widespread phenomenon of position effects (*4, 32, 33*), which range from position-effect variegation in *Drosophila* to X-chromosome inactivation in mammals. In each case, the affected genes are associated with a particular chromatin structure, are subject to a heritable form of transcriptional repression, and are replicated late in S phase (*33*). If these parallels are a manifestation of a common mechanism, then origins of replication may play a role in X-inactivation, position-effect variegation, and other heritable states of gene expression as shown here for transcriptional silencing in yeast.

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27. During the course of these experiments, the ratio of bubble signal to fork signal for a given origin varied among preparations. This variation was presumably due to differing amounts of breakage of bubble-shaped intermediates among preparations (*2, 19*). However, the relative ratio of forms was constant among different origins when the DNA samples were prepared in parallel. To control for these technical limitations, for each experiment, all samples were prepared from isogenic strains, manipulated in parallel, digested with the same restriction enzyme (or enzymes), analyzed on the same gel, and prepared from isogenic strains. The relative frequency of initiation among the sequences tested for origin function was estimated by Phosphor-Imager analysis (Molecular Dynamics). For maximum sensitivity, integrations were performed along a single line through the region of greatest signal for both bubble and fork forms. The ratio of bubble to fork signal was calculated from the integrated values and expressed relative to wild-type. For the *ARS* consensus mutant, no bubble form was detectable; therefore, a mock line was drawn that was comparable in position to the other lines. By this estimation the relative values for the test fragments were: wild-type (100%), 138 bp, and synthetic reconstructions (45 to 65%), and ars- (undetectable, estimated <5%).
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37. Two probes were used in these experiments. Both probes lie completely outside the regions that were mutated at *HMR-E*. One probe, to the **a1** gene, has been described (*38*). The other probe, whose relative position is indicated in the left side of Fig. 2, corresponds to the 0.7-kb fragment bounded by two Eco RI restriction sites. This fragment is located 0.2 kb internal to the indicated Hind III site.
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Cloning and Expression in Yeast of a Plant Potassium Ion Transport System

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A membrane polypeptide involved in K^+ transport in a higher plant was cloned by complementation of a yeast mutant defective in K^+ uptake with a complementary DNA library from *Arabidopsis thaliana*. A 2.65-kilobase complementary DNA conferred ability to grow on media with K^+ concentration in the micromolar range and to absorb K^+ (or $^{86}Rb^+$) at rates similar to those in wild-type yeast. The predicted amino acid sequence (838 amino acids) has three domains: a channel-forming region homologous to animal K^+ channels, a cyclic nucleotide-binding site, and an ankyrin-like region.

In contrast to animal cells, plant cells are generally exposed to low K^+ concentrations, often in the micromolar range. Growth in such conditions is made possible by high affinity K^+ transport systems in the plasma membrane (*1*). Biochemical efforts to purify the transporters are difficult, because of the low abundance of these transport proteins, and screening cDNA libraries with heterologous DNA probes has been generally unsuccessful in plants (*2*).

A mutant (*3*) of *Saccharomyces cerevisiae*, unable to grow on low K^+ medium and belonging to the same complementation group as the *TRK1* (*4*) K^+ transport system, was here complemented with a cDNA library made from *Arabidopsis thaliana* seedlings. An *Arabidopsis* clone (AKT1) was able to complement the yeast mutant and effect K^+ transport (Fig. 1). In the low (micromolar) K^+ concentration range, the K^+ (or $^{86}Rb^+$) uptake rates were similar in the wild-type and complemented yeast strains; both were much higher than in the mutant strain. When the K^+ concentration was increased to the millimolar range, the uptake rate reached a saturation plateau in the wild-type strain but in the complemented strain continued to increase with increasing K^+ concentration. The kinetics of the K^+ transport in the complemented strain were complex (Fig. 1B) and did not fit classical (Michaelian) saturation kinetics. The kinetics of K^+ uptake in plant roots are also quite complex (*1*) and may nevertheless result from the activity of a single transport system (*5*).

The capacity of the protein encoded by AKT1 to accumulate K^+ was verified by transferring complemented yeast into a K^+-free medium. After an initial loss of K^+ (*3*), which increased the external K^+ concentration to 10 μM, a net influx developed, decreasing the external K^+ concentration to 0.65 μM (*6*). Under these conditions, the cytosolic K^+ concentration was estimated to be 0.17 M (*7*). Thus, yeast transformed with AKT1 maintained a high K^+ accumulation ratio (K_{int}/K_{ext} approximately 2.6×10^5), which corresponded to an equilibrium potential difference (E_K) of about −320 mV. No estimate of the actual membrane potential difference is available, and it was not possible to determine whether the protein encoded by AKT1 mediated passive or active K^+ transport (*8*).

A Southern (DNA) blot of Eco RI–digested genomic DNA from *Arabidopsis* probed with the 2.65-kb AKT1 cDNA showed a single band. Northern (RNA) blot of total RNA from *Arabidopsis* indicated that a single 2.8-kb transcript hybridized with the AKT1 cDNA. The difference in length between the transcript and the cDNA may result from the loss of the polyadenylate tail or from cloning a cDNA incomplete in the 5′ upstream region (Fig. 2).

The AKT1 cDNA encodes a predicted peptide of 838 amino acids (MW 95.4 kD) (Fig. 2). No homology was found with the yeast *TRK1* gene product (*4*) or with K^+-transporting ATPases from bacteria and

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