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A Subset of Yeast snRNA's Contains Functional Binding Sites for the Highly Conserved Sm Antigen

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Autoimmune sera of the Sm specificity react with the major class of small nuclear RNA (snRNA)–containing ribonucleoprotein particles (snRNP's) from organisms as evolutionarily divergent as insects and dinoflagellates but have been reported not to recognize snRNP's from yeast. The Sm antigen is thought to bind to a conserved snRNA motif that includes the sequence A(U_{3-6})G. The hypothesis was tested that yeast also contains functional analogs of Sm snRNA's, but that the Sm binding site in the RNA is more strictly conserved than the Sm antigenic determinant. After microinjection of labeled yeast snRNA's into *Xenopus* eggs or oocytes, two snRNA's from *Saccharomyces cerevisiae* become strongly immunoprecipitable with human autoantibodies known as anti-Sm. These each contain the sequence A(U_{5-6})G, are essential for viability, and are constituents of the spliceosome. At least six other yeast snRNA's do not become immunoprecipitable and lack this sequence; these non-Sm snRNA's are all dispensable.

SMALL NUCLEAR RNA'S (SNRNA'S) are found complexed with proteins as small nuclear ribonucleoproteins (snRNP's) (*1*). In higher eukaryotes, five of the six most abundant snRNA's (U1, U2, U4–U6) are associated with one or more proteins that react with human autoimmune sera of the Sm specificity (*1, 2*). The functional importance of the Sm snRNP's was predicted by their extraordinary evolutionary conservation: human antisera immunoprecipitate snRNP's from species as divergent as frogs, insects, sea urchins, and dinoflagellates (*3, 4*). Indeed, four of these five U-snRNA's have now been shown to be essential for splicing of messenger RNA precursors (pre-mRNA's) in mammalian cell-free extracts (*5*) and the fifth (U5) is likely to be (*6*).

The evolutionary conservation of the Sm antigen does not seem to extend to lower eukaryotes, however, since the human sera were reported not to immunoprecipitate labeled RNP's from *Dictyostelium* or the yeast *Saccharomyces cerevisiae* (*3*). We have since shown that, like higher eukaryotes, yeast contains nuclear RNA's that possess the trimethylguanosine (TMG) 5′ cap that is specific to snRNA's (*7*). However, the large number of yeast snRNA species (≈24), their low abundance (≈10 to 500 molecules per cell), and their broad size distribution (≈120 to >1000 nucleotides) were unexpected (*7, 8*). Most surprisingly, the first three single-copy *SNR* genes we tested (*SNR3*, *SNR4*, and *SNR10*) were dispensable for growth, a result inconsistent with their participation in essential RNA processing reactions such as splicing (*9–11*).

The Sm antigen is believed to bind to a conserved motif in snRNA's, the sequence A(U_{3-6})G being embedded in a single-stranded region (*12*). The first time we encountered this consensus in a yeast snRNA was in the fourth gene we analyzed, *SNR7* (*13*). *SNR7* was also the first essential snRNA gene we identified (*11*). We thus hypothesized that yeast contains snRNA's that are the functional analogs of the Sm snRNA's, but that the Sm antigenic determinant has diverged too far to be efficiently recognized by human sera. Moreover, we predicted that Sm snRNA's would constitute only a subset of the yeast family and that these species in particular would be required for viability. To test whether snR7 contains a functional Sm binding site and, if so, to identify additional Sm snRNA species, we have taken advantage of observations that *Xenopus laevis* eggs and oocytes contain stockpiles of snRNP proteins and that microinjected snRNA's from HeLa cells or *Drosophila* can assemble with *Xenopus* Sm proteins to become immunoprecipitable with human anti-Sm sera (*14, 15*).

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The low abundance of the yeast snRNA's prompted us to choose an enriched source of snR7 for our initial experiments. Fraction I of the yeast splicing extract (*16*), which is generated by ammonium sulfate precipitation, is enriched by a factor of approximately 50 for a subset of RNA's that include snR7 (Fig. 1) (*17*). Fertilized *Xenopus* eggs at the one-cell stage were injected with pCp-labeled fraction I RNA or, as a control, *Drosophila* snRNA's (Fig. 1, lane b). After incubation, extracts were prepared and subjected to immunoprecipitation with anti-Sm antibodies (anti-Sm) (lanes d and m), anti-(U1)RNP antibodies (which is specific for U1 RNA–containing snRNP's) (lanes e and n), or a control nonimmune human serum (lanes f and o). Three RNA species from yeast were efficiently precipitated by anti-Sm (lane d); these are the two forms of snR7 [the longer form, $snR7_l$, contains a 35-nucleotide extension at the 3′ terminus

Table 1. Symbols: ++, quantitative precipitability by anti-Sm; +, strong precipitability; (+) weak (or possibly nonspecific) precipitability; −, no detectable precipitability. Putative Sm sites were identified strictly according to the proposed consensus A(U_{3-6})G (*12*); in the cases of snR7, -14, and -20, the regions containing the consensus sequence are predicted to be single-stranded. According to these criteria of sequence and structure, snR3 would not contain an Sm site and is thus unlikely to be the analog of U4 as we initially suggested (*9*). Disruption phenotype refers to the viability of haploid cells containing null alleles of the test *SNR* gene. These alleles are obtained by performing gene disruptions in vitro. Our typical strategy (*9–11, 23*) is to generate a gene disruption by inserting a second gene with an easily scored phenotype, such as *LEU2*. A linear DNA fragment flanked by sequences homologous to those in the yeast genome is then used to transform diploid cells (in this case, *leu2*−); this one-step gene replacement (*27*) produces cells heterozygous at the *SNR* locus. These diploids are then sporulated. The phenotype (viable or lethal) of spores containing the gene disruption (and therefore *LEU2*) will indicate whether or not the snRNA is essential. Not listed in this table are the other identified snRNA's, for which sequence and gene disruption data are not yet available (*7, 8*).

snR	Anti-Sm ppt	Putative Sm site	Disruption phenotype	Reference
$7_{l,s}$	+	AU_6G	Lethal	(*13*)
14	++	AU_5G	Lethal	(*18*)
17	(+)			
19	(+)			
20		AU_6G, AU_5G, AU_3G	Lethal	(*21*)
3	−	−	Viable	(*9*)
10	−	−	Viable	(*10*)
4	−	−	Viable	(*11, 19*)
5	−	−	Viable	(*19*)
8	−	−	Viable	(*19*)
9	−	−	Viable	(*19*)

(*13*)] and snR14. The specificity of this reaction is demonstrated by the absence of these signals from the control serum or anti-(U1)RNP (lanes e and f). Moreover, in a separate experiment with a monoclonal anti-Sm serum as well as a second patient anti-Sm serum, yeast RNA's contained in the immunoprecipitates were identical to those seen in Fig. 1. Analysis of the supernatants reveals that snR14 was quantitatively immunoprecipitated (lane g). Overexposure of this gel revealed faint signals with the mobilities of snR17 and snR19 in the anti-Sm immunoprecipitate (lane j); however, the snR17 signal was also present in the nonimmune control (lane k). Although it is difficult to evaluate the significance of this, the weak immunoprecipitability of snR17 and snR19 was reproducible.

We then asked if snRNA's other than those enriched in fraction I could become anti-Sm immunoprecipitable. However, when pCp-labeled total RNA from *S. cerevisiae* was injected into *Xenopus* eggs, the RNA was reproducibly degraded. In an attempt to overcome this problem, we enriched for the RNA's of interest by immunoprecipitating the starting RNA with antibody to trimethylguanosine (anti-TMG) before pCp labeling (Fig. 2, lane a). In addition, we turned to oocytes in the hope that RNA stability would be greater than in

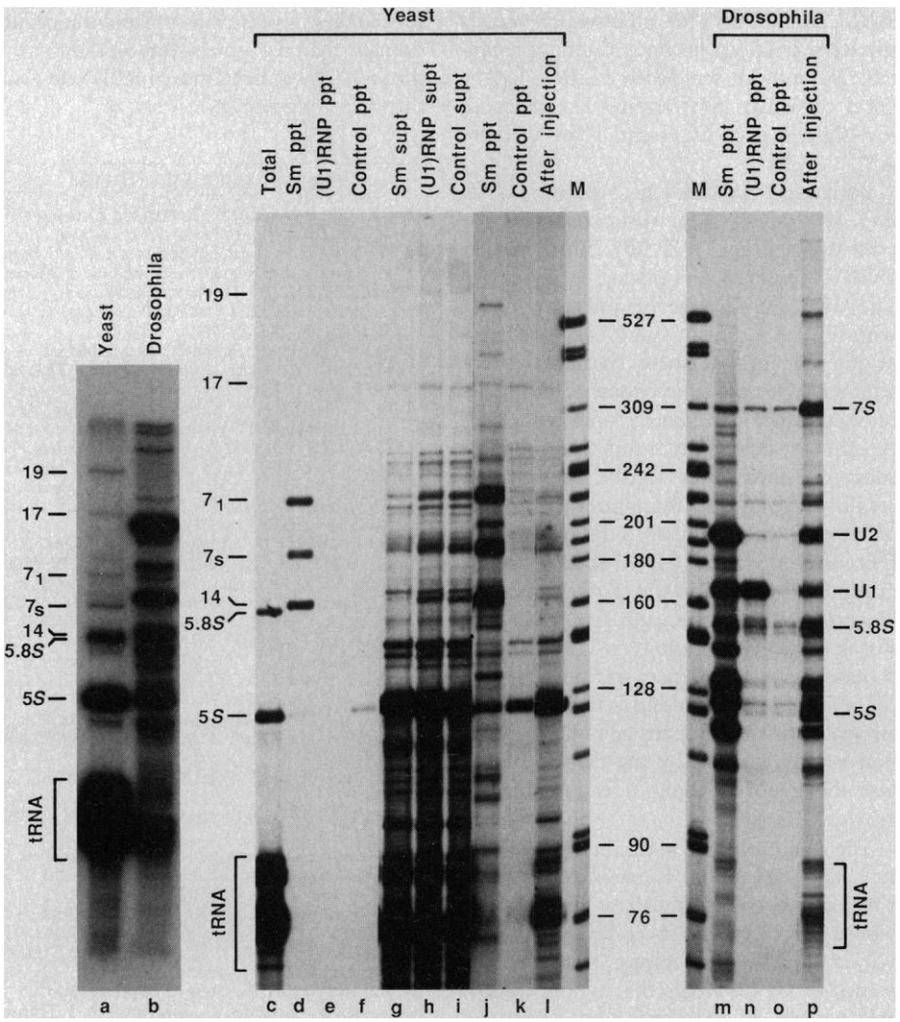

Fig. 1. Microinjection of snRNA-enriched fractions into fertilized *Xenopus* eggs. RNA was extracted with phenol from fraction I of a yeast-splicing extract (*16*) and labeled with pCp as described (*8*) (lane a); total yeast RNA is shown in lane c. Fraction I RNA was injected into five fertilized *Xenopus* eggs (≃20 ng per egg; ≃17,000 cpm per egg) and incubated as in (*15*). Extracts were prepared after 3 hours as in (*15*); a portion (10%) was directly phenol-extracted (lane l). The remainder was subjected to immunoprecipitation (*25*) with either a patient anti-Sm (lane d), a patient anti-(U1)RNP (lane e), or a control nonimmune human serum (lane f) (*26*); film was exposed for 48 hours. RNA extracted from supernatants (supt) of immunoprecipitates (ppt) is shown in lane g (anti-Sm), lane h [anti-(U1)RNP], and lane i (nonimmune); film was exposed 16 hours. Lanes j and k are the same as lanes d and f; film was exposed 2 weeks. Nomenclature of yeast snRNA's is as in (*8*). As a control, an snRNA-enriched fraction from *Drosophila* embryos was phenol-extracted, pCp-labeled (lane b), and injected as above (≃20 ng per egg; 4000 cpm per egg). RNA's contained in immunoprecipitates are shown in lane m (anti-Sm), lane n [anti-(U1)RNP], and lane o (nonimmune serum); RNA phenol-extracted directly after incubation is in lane p. The identities of Sm-precipitable species other than U1 and U2 are not known; presumably some bands are generated by degradation. End-labeled pBR325 Hpa II molecular size markers are shown (M). RNA's were analyzed by electrophoresis in a 6% polyacrylamide, 7*M* urea sequencing gel.

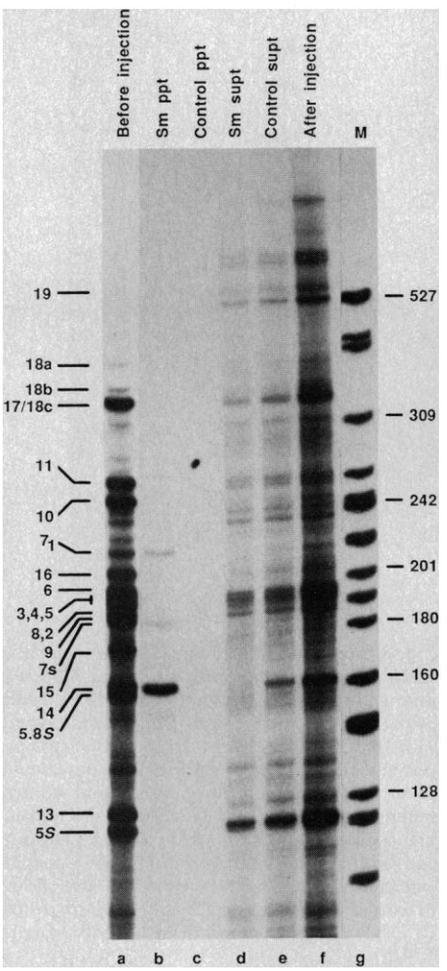

Fig. 2. Microinjection of snRNA's immunoprecipitated with anti-TMG into *Xenopus* oocytes. Total yeast RNA was first immunoprecipitated with anti-TMG antibodies and then labeled with pCp, as in (*8*). Labeled RNA was injected into 25 oocytes (≃0.16 ng of snRNA per oocyte; ≃11,500 cpm per oocyte) as described (*14*); lane a contains 10% of the starting material. After a 24-hour incubation, extracts were prepared and analyzed as described for Fig. 1. RNA's present in immunoprecipitates are shown in lane b (anti-Sm) and lane c (nonimmune control). Aliquots (10%) of RNA's remaining in supernatants are shown in lane d (anti-Sm), and lane e (nonimmune serum). RNA's phenol-extracted directly after incubation are shown in lane f. Abbreviation: M, end-labeled pBR325 Hpa II molecular size markers. In this experiment, the efficiency with which snR7 assembles with the Sm antigen relative to that of snR14 is less than seen in Fig. 1 (lane d). These differences can be accounted for, in part, by underrepresentation of snR7 in the starting material. Variations may also be due to differences in RNA stability in eggs and oocytes.

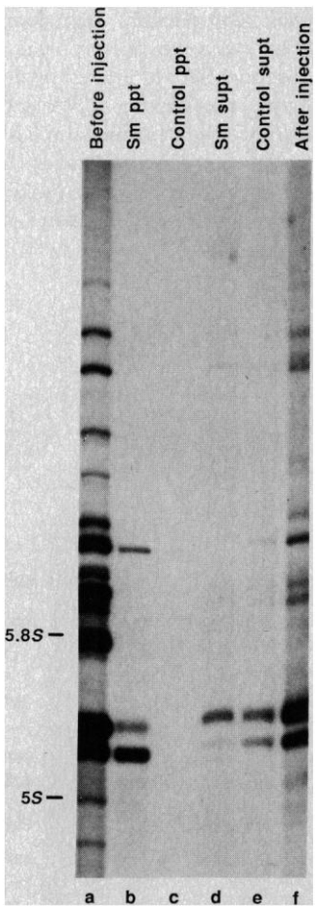

Fig. 3. Microinjection of anti-TMG–precipitable RNA's from *Aspergillus nidulans*. Total RNA was immunoprecipitated with anti-TMG, labeled with pCp (lane a), injected into 25 *Xenopus* oocytes (≈0.16 ng of snRNA per oocyte; ≈2500 cpm per oocyte) and analyzed as for Fig. 2. Lanes b and c show RNA's present in anti-Sm and control immunoprecipitates, respectively. RNA's remaining in supernatants are shown in lane d (anti-Sm) and lane e (nonimmune serum). RNA's phenol-extracted directly after incubation are shown in lane f. Positions of 5*S* and 5.8*S* ribosomal RNA's were determined from analysis of total RNA.

fertilized eggs. The results were virtually identical to those seen in Fig. 1; snR7 (both forms) and snR14 were specifically immunoprecipitated (Fig. 2). As before, snR14 appeared to have been quantitatively removed from the supernatant.

The absence of other snRNA species in the immunoprecipitate may be attributed to several causes. In many cases (such as snR10), sufficient RNA was present in the supernatants for us to argue that these RNA's were not being efficiently or stably assembled with *Xenopus* Sm antigen. In contrast, we cannot draw conclusions about species that are highly unstable (such as snR18) or those that were present in only low abundance in the starting material [such as the recently described class of minor snRNA's (*8*)]. Other potential sources of "false negatives" include the inability of the RNA to assume the appropriate secondary structure and competition of other binding factors for the Sm site.

The identification of a subset of snRNA's that assemble with the *Xenopus* Sm antigen has allowed us to make a number of predictions, several of which have been borne out (Table 1). First, *SNR14* has been cloned and sequenced (*18*); the gene encodes an RNA that, like snR7, contains a good candidate Sm binding site. Moreover, we have sequenced three additional *SNR* genes (*SNR5*, *SNR8*, and *SNR9*) (*19*), all of which encode species that fail to become immunoprecipitable by anti-Sm sera; each of these lacks a good consensus Sm binding site. Second, the *SNR14* gene is essential for viability (*18*).

Both snRNA's that efficiently reassemble into Sm snRNP's are components of the yeast spliceosome (*17, 20*). Furthermore, *SNR7* is required for splicing in vivo (*13*). The weak immunoprecipitability of bands with the mobilities of snR17 and snR19 is of interest in that these species also cosediment with the spliceosome (*17*). Nucleotide sequence and gene disruption data will be required before the significance of these additional correlations can be assessed. We are unable to test the immunoprecipitability of another snRNA that is present in the spliceosome [snR20 (*17*)] because of its extremely low yield in the starting material. Since this RNA [also called lsr1 (*21*)] has strong sequence homology to U2 RNA and contains several consensus Sm binding sites (*21*) (Table 1), it should have the capacity to associate with the Sm antigen. This prediction is strengthened by the demonstration that the gene encoding it is essential for growth (*21*).

The extent of the correlation between Sm type and essentiality remains to be seen. That all known mammalian Sm snRNA's whose functions have been assessed are involved in RNA processing reactions presumed to be essential (that is, pre-mRNA splicing and 3′ end generation) (*5, 6, 22*) suggests that all Sm snRNA's are likely to be required for growth, as are snR7 and snR14. Each of the six yeast snRNA's that lack apparent Sm binding sites is dispensable for viability (*9–11, 19*). It is possible that they play potentially overlapping (*9*) or even nonessential functions in highly complex processes such as ribosomal RNA maturation (*23*).

Our finding that only a minority of the yeast snRNA's are of the Sm type is in striking contrast to the current picture of snRNA's in higher eukaryotes. Here only a single example of a non-Sm snRNA, U3 (*24*), is known. Our work predicts that other non-Sm snRNA's remain to be discovered in metazoans. In any case, the predominance of non-Sm snRNA's is not restricted to yeast. Our analysis of snRNA's from another representative fungal species, *Aspergillus nidulans* (Fig. 3), reveals that here, too, only a small subset of TMG cap-bearing RNA's become immunoprecipitable upon injection into *Xenopus* oocytes.

Conservation of the ability to bind the Sm antigen extends across the broad evolutionary expanse from humans to fungi. Our studies predict the existence of an Sm protein analog in yeast; presumably the protein domain that recognizes the binding site in the snRNA has been more highly conserved than the antigenic epitopes.

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Lunar-Modulated Geomagnetic Orientation by a Marine Mollusk

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Behavioral experiments indicated that the marine opisthobranch mollusk *Tritonia diomedea* can derive directional cues from the magnetic field of the earth. The magnetic direction toward which nudibranchs spontaneously oriented in the geomagnetic field showed recurring patterns of variation correlated with lunar phase, suggesting that the behavioral response to magnetism is modulated by a circa-lunar rhythm. The discovery of a magnetic sense in a mollusk with giant, reidentifiable neurons provides a unique opportunity to study the cellular mechanisms underlying magnetic field detection.

ALTHOUGH THE MAGNETIC FIELD OF the earth is known to influence the orientation of a variety of organisms (*1*), the neurophysiological mechanisms underlying magnetic field detection in metazoans have not been established. It has been hypothesized that ferrimagnetic material functions as a transducer for a magnetic sense in several animals (*1, 2*). However, primary magnetoreceptors have proven difficult to isolate, and direct neurophysiological evidence implicating ferrimagnetic particles in the detection of magnetic fields has not been obtained.

Electrophysiological analysis of the neural mechanisms underlying magnetoreception in vertebrates has been impeded by the small size of vertebrate neurons, difficulties in reidentifying individual cells, and the complexity of the central nervous system. Here we report a behavioral response to ambient magnetic fields by the marine mollusk *Tritonia diomedea,* a nudibranch that has large, reidentifiable neurons and a relatively simple nervous system accessible to electrophysiological studies (*3*). In addition, we present evidence of a novel pattern of orientation based on magnetic field detection and related by an unknown mediator to lunar phase.

When tested in the laboratory, a variety of invertebrates spontaneously orient to context-irrelevant stimuli (*4, 5*). Experimental manipulation of such spontaneous orientation has been used to demonstrate sensitivity to ambient magnetic fields (*5*) and to polarized light (*4*). In an initial 4-day experiment, we therefore examined the orientation of *T. diomedea* maintained in darkness under two ambient magnetic field conditions. On nights 1 and 3 of the experiment, nudibranchs were tested in the magnetic field of the earth (*6*). On nights 2 and 4 the horizontal component of the earth's magnetic field was canceled (*7*) by using a Rubens' coil system (*8*) to generate a field equal in intensity to the horizontal component of the geomagnetic field but opposite in direction (*9*).

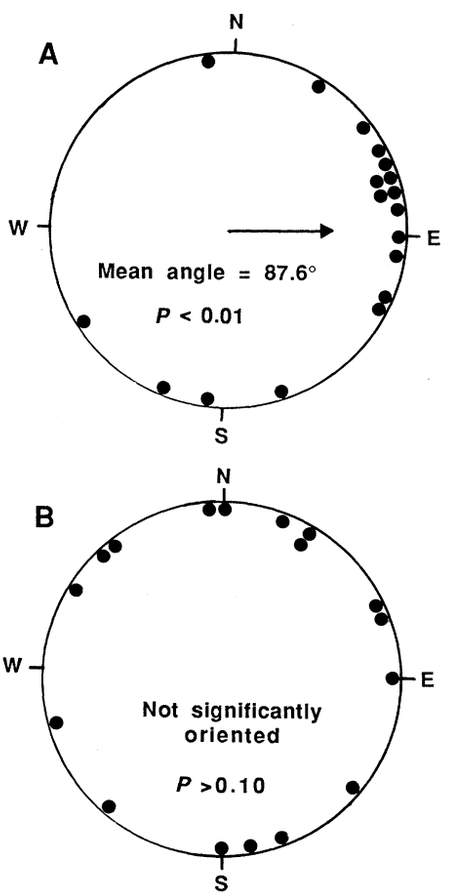

Fig. 1. Orientation of *Tritonia diomedea* under two ambient magnetic field conditions. (**A**) Orientation in the geomagnetic field. The group is significantly oriented [$n = 18$, $r = 0.58$, mean angle = 87.6°, $P < 0.01$, Rayleigh test (*10*)]. (**B**) Orientation in a field with a canceled horizontal component. The distribution is indistinguishable from random [$n = 17$, $r = 0.19$, mean angle = 29.0°, $P >> 0.10$, Rayleigh test (*10*)].

In the earth's field, the orientation of the animals was significantly [$P < 0.01$, Rayleigh test (*10*)] nonuniform with a mean angle of 87.6° (Fig. 1A). In contrast, animals tested in the canceled horizontal field showed orientation statistically indistinguishable from random (Fig. 1B). A comparison of the two distributions with the Watson test (*11*) indicated that they are significantly different ($U^2 = 0.199$, $P < 0.05$). These data suggest that the eastward orientation observed in the geomagnetic field was mediated by magnetic field detection and that eliminating the horizontal component of the geomagnetic field impaired the ability of the animals to orient. We emphasize that our measurements were of body axis alignment only and not of directional movements [an animal on the western side of the tank could be oriented eastward (*12*)].

The results of this initial experiment encouraged us to examine in greater detail the orientation of *Tritonia* in the natural magnetic field of the earth. Measurements of body axis alignment conducted sporadically over a period of 4 months, however, indicated that the animals did not always orient toward the east (*13*).

Initially we did not perceive any pattern in the directional variation and unsuccessfully sought to identify variations in experimental procedure that could account for it. Several reports, however, have suggested a relation between geomagnetic orientation and lunar phase. Homing pigeons, for example, utilize geomagnetic cues in orientation and show an apparent lunar rhythm in their initial bearings from some release sites (*14*). Mud snails and flatworms show behavioral responses to magnetic fields that reportedly vary subtly with lunar phase (*15*), as does the fly *Drosophila* (*16*). In view of these reports, we plotted the mean angles of orientation for groups of nudibranchs as a function of day of the lunar month (Fig. 2). The results of circular correlation analysis (*17*) indicated the two parameters were sig-

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