| Name | Access number | Amino acid sequence [a] | Predicted peptides |
|---|---|---|---|
| Che-pp1 | KX496947 | MERKILACLFLLIVLLNLNDGKNIAILIEPDDNLASELEWLGSDMTDSHSLNAGAWPRPGDARSSHD AWPRPGKREFYGNEMFEKRPFPGQQMQFSWPRPGKKRTKEDTWPRPGKRESYSEGDMDSRSGALRRS EEKETNEDEKLENAWPRPGKREFYASRKMDVRPRGGRDSKSHKISKRNSEAISNDEIDMMIREEAWP RPGKRDYHMLSATRPRGGKDARPRGGKDSSRPRGGKNAKPRGGKDSVRPRGGKKDSWPRPGKDAFVK EINGSRPRGGKDASKWPRPGKKDLK | 7 x WPRPa<br>6 x RPRGa<br>1 x KPRGa |
| Che-pp2 | KX496948 | MKIYFGCLFVILSVNQIGCYPSSNQNSERELVRRIYKTVHPNPHYQVNEIQRVKEALKRRVLENVHR VDSLKASLKRVLGQDAGNGFHMSSSKIFQKKRSKARLPHSYMFRKRQNSPGALGLWGREVEAPGDIG PPGIWGDVVPDETRKDKPGAVQGLWGKDERVIRALLKTLKR | QNSPGALGLWa<br>KPGAVQGLWa |
| Che-pp3 | KX496949 | MNCVLIFLVLFLANNVYSASLTREEDALVTKLLDTIEKRDAVPRLGKREVPRLGREIEDVPRLGREI ELVPRLGREAEEVPRLGREAEEVPRLGREVELVPRLGREVELVPRLGREVEVPRLGREVPRLGRGID EVPRLGREVPRLGREVLEVPRLGREVPRLGKREIPRLGKREVPRLGRREVPRLGREASTYDLKQLYNQ LKREVSNDMIEAEIKEEKRVLKAFDLGTRGLILRRIGDKLNKDGFSDQKRDMNEKESSRPFLHVKRT NLLSLIEKLTSEE | 15 x VPRLa<br>1 x IPRLa |
| Che-pp4 | KX496950 | MNLLVSIPVICAIVLKLTESAPISNVRKIGSNELLLKLTVSDLAKLLSRLQNVHEDGHKEDLNKVSV EGMIADYLDEKQYKDRPRYGKDLKEASRPRYGKEMSEGGNHNVIEQLIEKLVNQSSESDTKDDGNIK SDGKVDNLVSLLHGLDEEKEWPRPGKDWPRAGKDWPRAGKDWPRAGKDWPRAGKDWPRPGKDWPRPG KDQPNGIARGGKKSLGEILSDLMSKRPRYGKDESSTNGPSYQYTLEDMMTEIISGDRPRYGRKSEAS RPRYGKESADKTLEEALSNLGTKSTKKDTSSRSSLARGGKKRGVEDLILEYIENHLDDKKDYESARK DTPK | 5 x RPRYa<br>5 x WPRAa<br>2 x WPRPa IKSDa<br>IARGa<br>LARGa |
| Che-pp5 | KX496951 | MELKYFLASFIFVIIAIQLASCSSKAEEYKQMKKEVDGLLKEIVSQENAKQHTSEKKSSQWLNGRFG KRQLVSGRFGRELKQWLNGRFGREATEQWLNGRFGKREDDQWLNGRFGREVEQWLNGRFGREAAEQW LNGRFGKRSANQWLNGRFGKRSADQWLNGRFGKRSADQWLNGRFGREASADQWLNGRFGREAADQWL NGRFGREAKEQWLNGRFGREVEQWLNGRFGREAGQWLNGRFGREMGQWLNGRFGREADQWLNGRFGR EADQWLNGRFGRDAAPLAARYGDEPAHVESQTIAAPEEAKPKIVAAVKVVKKPVAVSE | 17 x QWLNGRFa |
| Che-pp6 | KX496952 | MARESLLFFLLALHCCEAFYNGDVPRRRSEASRLLMAKDASQKHASSDTSRLLFGKDAPQRHVSSDS RLLFGKDAPQRHVLSDTSRLLFGKDAPQRHVSSDSRLLFGKDAPQRHISSDSRLLFGKDAPQRHVSS DSRLLFGKDAPQRHVSSDTSRLLFGKDAPQRHVSSDTSRLLFGKDVPQRPAGGSDSRTSYTAHLMPT LGKDEYVNALKERLLNDYRMKLLQQQRQQQHQQQEDDDELYFDHSFRRGNNPSASAFRRRYSDQTGQ PLSGSRDQKVNQDENARDTLEKKQQASLDQTKEELKRSLLKDFYKKMITEKREKQEFAKKSDAPVSN DFDDEILRHLVEFKLKKEDPMKRMRR | 8 x SRLLFa |
| Che-pp7 | KX496953 | MRFCSWTNLFLLGITCLCLTNGMPNKQHVRNKKNLIDNTVKMADHGKTLVKKSAHPMKIKDVSKKST GGGSDIANSDDTFDRAADGTDNSLYGRQEKEQGTENSGVGKFEGPPCRWGCGKREAGIDGPPGRWGG RKRGMRRVGPPGRWGGRKRGELPGRWGGKKRGELPGRWGGKKRSELPGHWGGKKRSELPGHWGGKKR SEIPGRWGGKKRSEIPGRWGGKNRSELPLGWSQKEGNQRPPSKET | 2x LPGRWGa<br>2x GPPGRWGa<br>2x IPGRWGa<br>2x LPGHWGa |
| Che-pp8 | KX496954 | MLSSETTIRIFCFFIAVGFAVGSSSPEEEGQLLHVKRETWLNPGFDSMLHRRESQELLNRPRPGRRE LFDLMNQDSILKKRALLHRPRPGRREFLFRNQGLDSMLHKRAMLNRPRPGRREFLFRNPGLDSMLHKRG QEFLSGPRPGREIRPRPGRREERHPDSMLHRRSSDTDLDYQHLLRNPRPGRREFLFRPRPGHRELQHLD SLLHRRSEEMWGRPRPGKREVYYEDDGRSEDEKLLRVLDELKRDIIDELWDRFQN | [b]<br>6x --RPRPa<br>1x --GPRPa<br>1x --NPRPa |
| Che-pp9 | KX496955 | MYLSVGFLLFLCHQFQDTHGLSIRGPNDAQQLINSHGENDLPSGGMWETAKSQAMETYRKDSRRGIP KRGRSSFLAIGKKDDSLSLGSKMKKSLESPSLSVWRRGDSLHSILRVNPSLGIWKRGDSLMRRFGRK NFGKDNSRRSLVREIENDAFGMNRKDEFPSPGMWEKAKSQYFGKREFRNSILGKHGKDEFPSPAMWE KAKNQYFGKRDIRNSILGKHGKDEFPSPAMWEKAKNQYFGKGR | 3x --QYFa<br>2x LGKHa<br>SLMRFa |
| Che-pp11 | KX496956 | MDQSLSSILLLLLCCWVALTTCMSVQRKEAGDALSALDKENAKKSANSITEELARNLMEHLYDEIRKR SNSNEETISNFRRASSDTHRQQQAPKGLWGRELQPGNPPGLWGREASEAENTDSNDGPIPGMWGRRE ADDKNAHEKFQ | QQQAPKGLWa<br>QPGNPPGLWa<br>GPIPGMWa |
| Cpa-pp1 | KX496957<br>KX496958 | MISKAIIVYSLVLLILPVYSKFPKSRFSIRGNQLDNGYPRGEIPKIKPDMKDRERSNLLMKFAEALR ILRTNQNYHDGKPYSKDIEEDFRKISSNRFNIRPRPGKRFLKNKFAACDEHFANE | RPRPa |
| Cpa-pp2 | KX496959 | MAMNITFISFICFAISIILLNVQSAPLDDIRERSIEHEMAQNQRESLVHLLTLGYSLGARDDSNTLS KLITYVDNLKDDVWNTDSEGCRNIETTLYSDLLSQPNYDLTDLESRELDTYENERPTAGKRFSSMNI IDNLSFRPRAGRRDIRPRAGRREFRPRAGKRYLAESESFDAYRPRAGKREDLRPRAGKRDEDNEKMRD TANRQSQREVLKRNSIYKELMTANESNERPRAGKREDETETDEGKHDMSTFLEINSDKREERPRAGKKE LRPRAGKKELRPRAGKKE | 9x RPRAa |
| Cpa-pp3 | KX496960 | MKTTMLYHTVLCICVINIYGNGKFVYTENAIDEKELTDLKDLQLDAESNQIQSRITKDLSRILADKI YNQLKESTNTEFKKNLDLFQFQPEIGSFNGKRPPLPKPPGLWGKDVHSRFQIDSHDISIKSSGDISK REAEKRKLKNNLKSDDASKYADYINMDNKRSEILNGPPGLWGREAEKLNGPPGLWGRDEKVNDALNG KLGKVHEPRDLLKRENGKVNGPPGLWGREIGKRNGPPGLWGRDAEMNNGPPGLWGRDAEKVNGPPGL WGRDIGKINAPSSLQDHTFEKNRGPPGLWGREIEKVNGPPGLWGREIEKNNGPPGLWGREIGMNNGP PGLWGRDFTTIKENGDNDATRKEKPTDTKMENSDKKGGFGQKDDSKYNAKWKRNERNLLKDRNQIET QKRESSRKESSADKQIKNRNSKDSKTAKKDSRNDVTIDNIFPWHKSKHDDNTASNLRDIIENEF | 10x NGGPPGLWa |
| Cpa-pp4 | KX496961 | MNLILPIFASILLCLADSASITTKKDSENDVERQIETLLNELLEGADNNLYDKKEVDQWLKGRFGREE SSDQWLKGRFGRDADQLLKGRFGREAEQWLKGRFGREAGDQWLKGRFGREAEQWLKGRFGREAGQWL KGRFGREAEQWLKGRFGREVEQWLKGRFGRETEEAEQWLKGRFGRDAITKDSNPLLRGRYGREESESE VTENAPKQLDRGRYGREQWLKGRFGREAQSENETKKEIDNANKKVDVNAKKEVSKTAM* | 9x QWLKGRFa 2X RGRYa |

[a] N terminal secretion signal sequences are highlighted in pink. Probable mono- or di-basic cleavage sites, highlighted in blue, are preceded by Glycine residues (yellow) that would be converted to C terminal amides. N terminal protolysis limits of each liberated peptide (best estimates in grey) during precursor cleavage in cnidarians are difficult to predict, but are often associated with additional basic or acidic residues (red).

[b] N terminal limits of possible peptides generated for pp8 are particularly hard to predict, but all have the C-terminal sequence PRPa

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**Figure S1. Amino acid sequences of putative precursors of amidated peptides identified from *Clytia hemisphaerica* (Che) and *Cladonema pacificum* (Cpa) transcriptome data.**

N terminal secretion signal sequences are highlighted in pink. Probable mono- or di-basic cleavage sites highlighted in blue are preceded by Glycine residues (yellow) that would be converted to C terminal amides in the final peptides. The limits of proteolysis on the N terminal side of each liberated peptide during precursor cleavage in cnidarians are variable and difficult to predict but are often associated with additional basic or acidic (red highlighted here) residues. Genbank Accession numbers for each peptide precursor sequence are indicated in parentheses.

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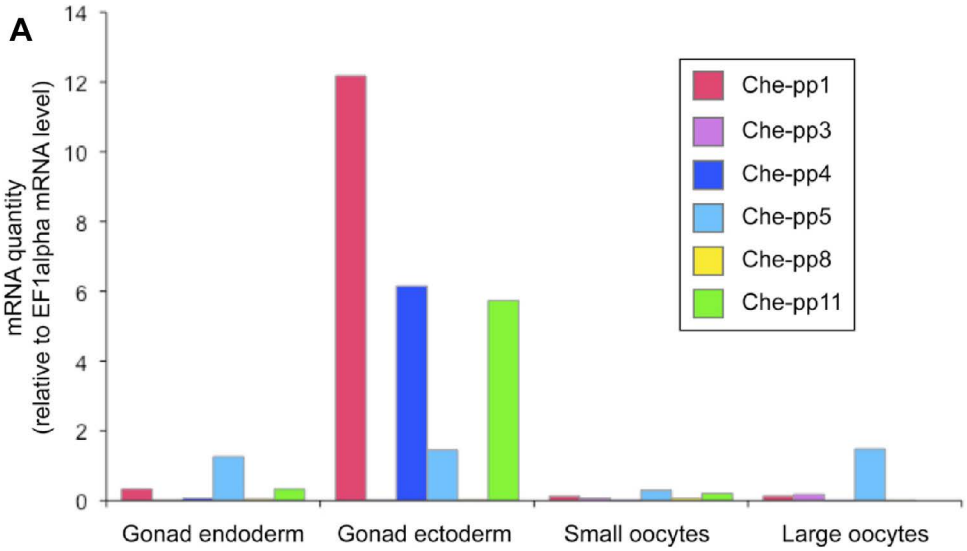


**B**

| | | |
|---|---|---|
| EF1alpha | Forward | 5' TGCTGTTGTCCCAATCTCTG 3' |
| | Reverse | 5' AAGACGGAGTGGTTTGGATG 3' |
| Che-pp1 | Forward | 5' GCGTCGATCTGAGGAAAAAG 3' |
| | Reverse | 5' AACGTCCATCTTCCTGCTTG 3' |
| Che-pp3 | Forward | 5' CTGAAAGCGTTCGATCTTGG 3' |
| | Reverse | 5' CACATGCAAGAATGGTCTGC 3' |
| Che-pp4 | Forward | 5' CGTCAAGTCCGTCATCATTG 3' |
| | Reverse | 5' GCTGACTCATCATCTTTCTTGTC 3' |
| Che-pp5 | Forward | 5' ATCCTGCTCATCCAAAGCTG 3' |
| | Reverse | 5' AACTCACGGCCAAATCTACC 3' |
| Che-pp8 | Forward | 5' GGACGAGAAATAAGGCCAAG 3' |
| | Reverse | 5' TGATCGTCGATGCAGTAAGG 3' |
| Che-pp11 | Forward | 5' TACTATGCTGTTGGGTCGCC 3' |
| | Reverse | 5' CTTCAGTAATGCTGTTTGCGCT 3' |

**Figure S2. Quantification of neuropeptide transcript levels between gonad tissues.**

A) Quantitative RT-PCR analysis of neuropeptide precursor expression in manually separated tissues from *Clytia hemisphaerica* gonads confirmed that Che-pp1, Che-pp4 and Che-pp11 are the 3 main peptide precursors expressed in the ectoderm. Q-PCR was run in triplicate and EF1alpha used as the reference control gene. B) Sequences of forward and reverse primers used for each gene.

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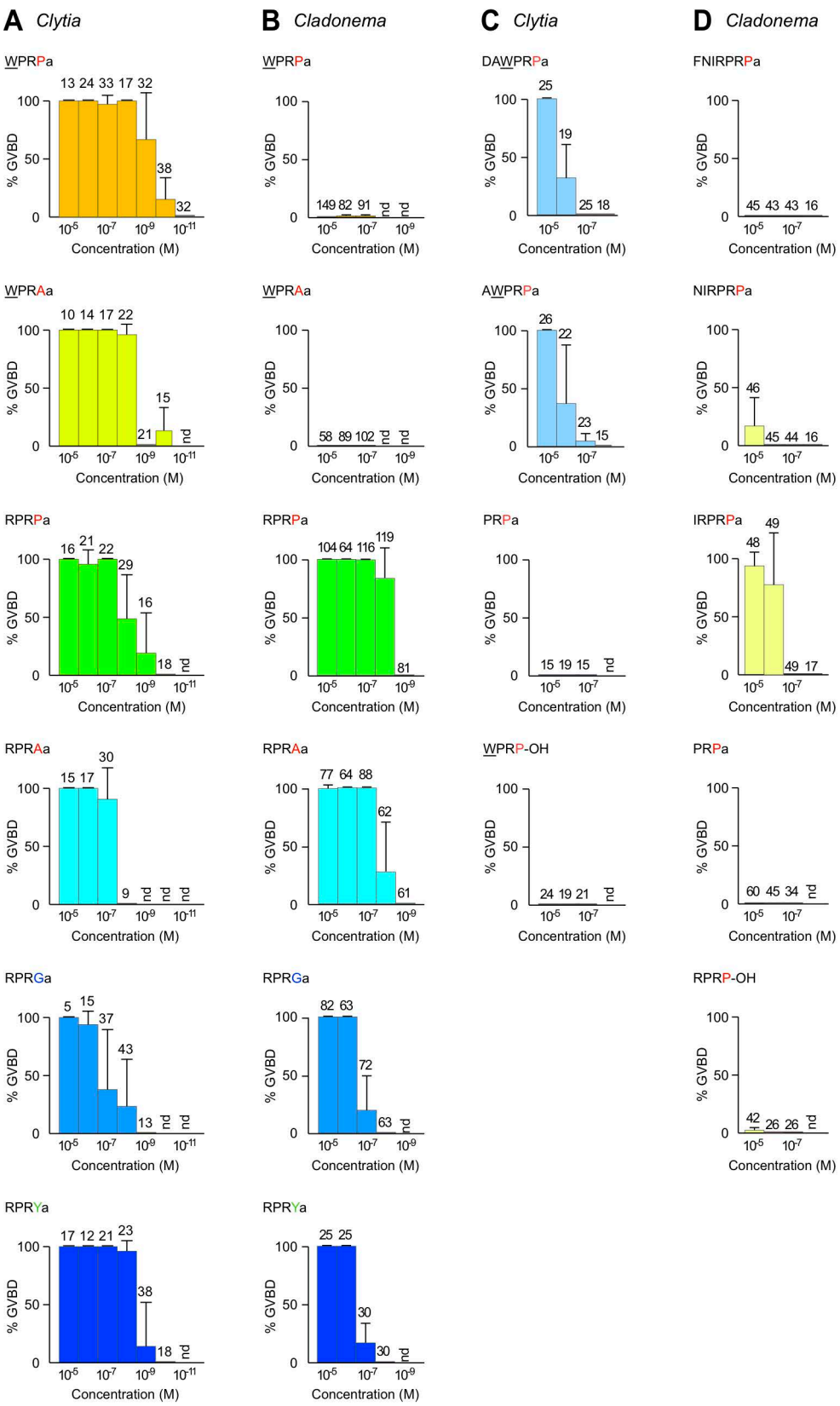


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**Figure S3. MIH activity of synthetic amidated peptides.**

*Clytia* (columns A and C) or *Cladonema* (columns B and D) oocytes were incubated in SW drops containing synthetic amidated peptides as indicated, and GVBD scored after 2 hours. The numbers of oocytes tested is shown above each bar. Black lines indicate standard deviation between experiments. Simplified representations of these data are shown in Fig. 2C.

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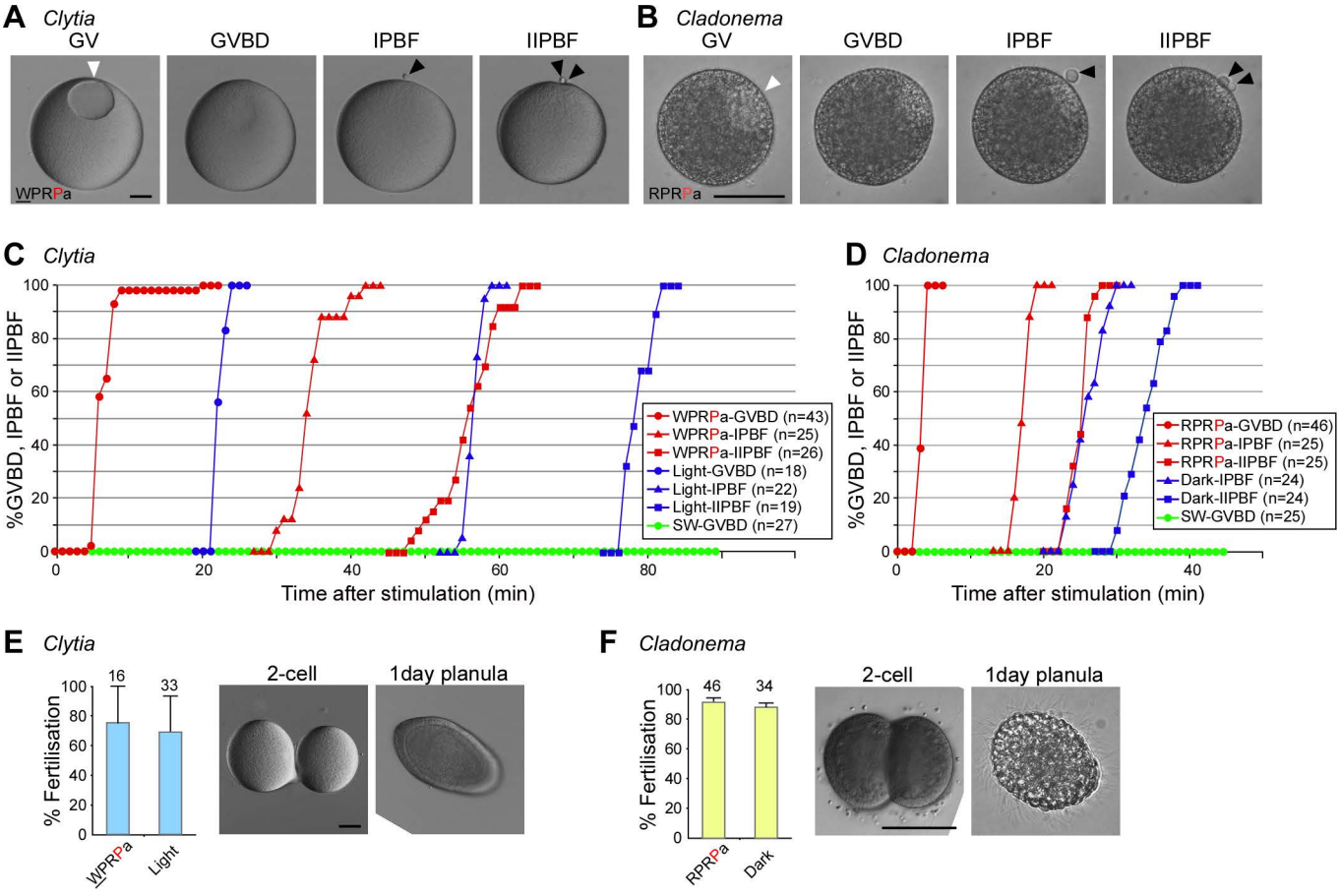


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**Figure S4. Synthetic tetrapeptides induce normal oocyte maturation.**

A, B) Images of *Clytia* and *Cladonema* oocytes respectively, incubated in $10^{-7}$ M W/RPRPamide as indicated, undergoing successive steps of meiosis: GVBD; First polar body formation (IPBF); Second polar body formation (IIPBF). C) Time course of *Clytia* oocyte maturation. The percentages of oocytes undergoing GVBD, IPBF, and IIPBF after application of $10^{-7}$ M WPRPamide or SW alone (control), or light stimulation are indicated as a function of time. The time of IPBF and IIPBF after light stimulation was determined in oocytes isolated from light-stimulated ovaries following GVBD. WPRPamide- and light-induced meiotic maturation progressed at a similar speed, although there was a lag time of 15-20 minutes. D) Time course of *Cladonema* oocyte maturation. Meiotic maturation in isolated oocytes treated with $10^{-7}$ M RPRPamide was advanced by about 10 minutes compared to that induced by dark treatment of gonads. At 11~12 minutes after dark initiation, oocytes just undergoing GVBD were isolated from the gonads for subsequent observation of IPBF and IIPBF. E) Fertilisation rates for *Clytia* oocytes matured by incubation in $10^{-7}$ M WPRPamide were equivalent to those released from gonads stimulated by light. Numbers of gonads tested and standard deviation between experiments are shown for each bar. They went on to undergo normal cleavage divisions (center panel) and form swimming planula larvae (right panel). F) Equivalent fertilisation success documented for *Cladonema* eggs incubated in $10^{-7}$ M RPRPamide. Scale bars: 50 µm. White and black arrowheads indicate GVs and polar bodies, respectively.

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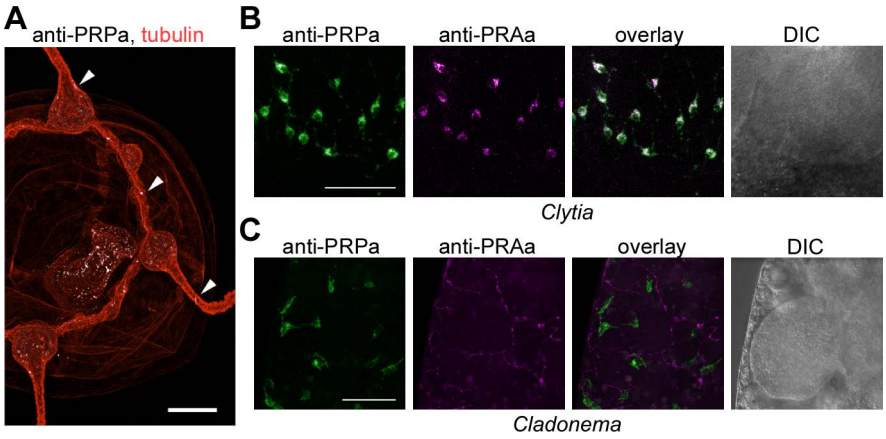


**Figure S5. Immunodetection of MIH in *Clytia* jellyfish and in *Clytia* and *Cladonema* gonad ectoderm.**

A) Confocal image (summed Z stack) of a *Clytia* baby jellyfish following immunofluorescence performed with anti-PRPamide and anti-tubulin. Arrowheads point to MIH-immunopositive cells in the nerve ring and tentacles. B, C) Epifluorescence images of gonad ectoderm following double immunofluorescence performed with anti-PRPamide and anti-PRAamide antibodies as indicated. Overlaid images are shown in the third panel of each row. In *Clytia* gonads (B) these decorated a single cell population, whereas in *Cladonema* (C) the two peptides were detected in distinct cell populations. Scale bars: 50 µm.

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