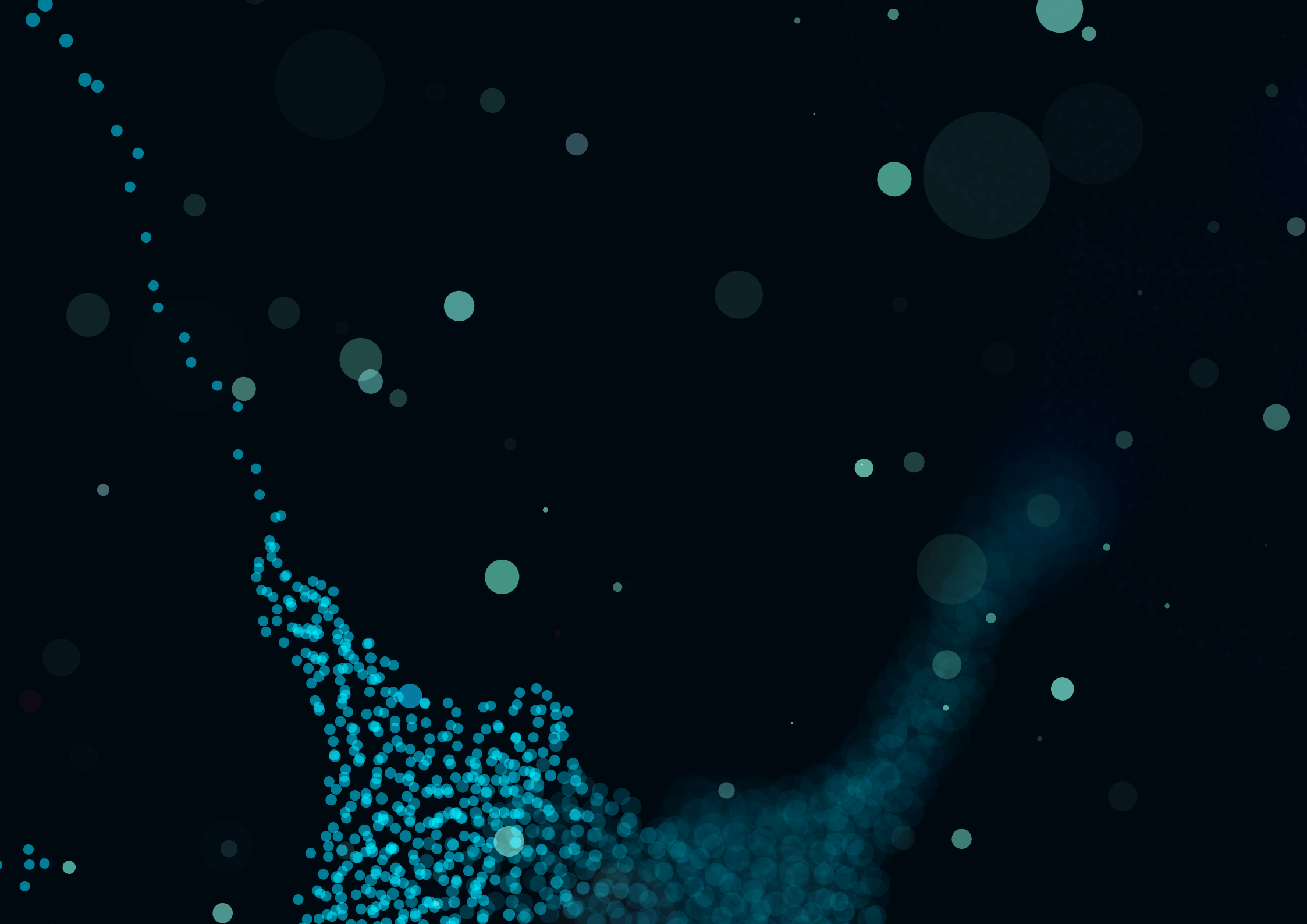


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EXECUTIVE SUMMARY

BRIEF OVERVIEW OF THE COMPANY



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Are focused on delivering quality, service, and at very competitive prices.



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At Formedium, we specialise in providing blending technology for the manufacturing of industry-standard media and custom nutrient formulations. Our team is highly skilled and agile, enabling us to swiftly respond to the evolving needs of our clients in microbial, cell, and bacterial growth research.

We combine advanced blending techniques with a deep understanding of product development to deliver tailored solutions that support optimal growth conditions for a wide range of scientific applications. Whether you need standard formulations or bespoke blends, we ensure the highest quality, consistency, and precision in every batch.



ABOUT FORMEDIUM



Ian Hodge

Specialised in the chemical and biochemical sectors with a focus on laboratory chemicals, custom synthesis, and research products.

Leadership experience in distributing laboratory chemicals and equipment, as well as managing the sales of specialised biochemical products.

Currently leading a company that manufactures custom bacteriological media for research, with expertise in yeast, E. coli, and fungal systems.

Passionate about science, supporting research projects worldwide.



Suzie Hodge

Extensive experience in field trials, administration, and consultancy.

Early career involved managing field trials in the chemical industry and working as an administrator for a major research and development department.

Later transitioned into consultancy, specialising in policy development for workplace substance abuse.

Since 2002, a key leader at Formedium, where her passion and commitment are unmatched.

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Senior leadership



Ian



Suzie

Office Team



Anne



Becca



Alex

Laboratory and Manufacturing team



Dave



Tom



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2

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Transplanting human infant gut microbiome species into Galleria mellonella

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Short Report

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Genetics

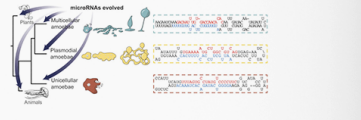


Evolution of microRNAs in Amoebozoa and implications for the origin of multicellularity

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Abstract
MicroRNAs (miRNAs) are important and ubiquitous regulators of gene expression in both plants and animals. They are thought to have evolved convergently in these lineages and hypothesized to have played a role in the evolution of multicellularity. In line with this hypothesis, miRNAs have so far only been described in one unicellular eukaryote. Here, we investigate the presence and evolution of miRNAs in Amoebozoa, focusing on species belonging to Apicomplexa, Phylum and discoidal taxonomic groups, representing a range of unicellular and multicellular lineages. miRNAs that adhere to both the stringent plant and animal miRNA criteria were identified in all examined amoebae, expanding the total number of prokaryote harbouring miRNAs from 7 to 15. We found conserved miRNAs between closely related species, but the majority of species feature only unique miRNAs. This shows novel gain and/or loss of miRNAs in Amoebozoa, further illustrated by a detailed comparison between two evolutionary closely related dictyostelids. Additionally, loss of miRNAs in the *Dictyostelium discoideum* *dm1* mutant did not seem to affect multicellular development and, hence, demonstrates that the presence of miRNAs does not appear to be a strict requirement for the transition from uni- to multicellular life.

Graphical abstract



Introduction
miRNAs (miRNAs) are small, ~21 nucleotide (nt) non-coding RNA (ncRNA), which control gene expression to create intricate regulatory networks (1). The vast majority of miRNAs have been identified in animals and plants (2–7). In both these lineages, miRNA biogenesis involves transcription of larger primary, hairpin structured precursors (pri-miRNAs) that are processed to pre-miRNAs before being matured into miRNAs (8). This process is performed by a machinery derived from the ancestral RNA-interference (RNAi) machinery that dates back to the last eukaryotic ancestor and protects cells from viruses and mobilization of transposons (9–13). Once processed to mature miRNAs, these RNA then guide the

specific genes to be regulated based on binding to their target mRNAs via sequence complementarity, thereby guiding the RNA induced silencing complex (RISC) to perform translational silencing and/or induce target RNA degradation (14). Even though miRNA maturation and formation of the RISC involve a number of associated proteins – to some extent differing between animal and plants – two proteins, Dicer and Argonaute, are central to a functional RNAi machinery regardless of organism. Dicer binds to miRNA hairpin structures and cleaves out double stranded miRNAs, consisting of a mi-mip and mi-mp duplex. Argonaute are the effector proteins, which select the miRNA (either the mi-mip or mi-mp) from the duplex. The mature miRNA then guides the

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Equipping *Saccharomyces cerevisiae* with an Additional Redox Cofactor Allows F₄₂₂-Dependent Bioconversions in Yeast

Misun Lee and Marco W. Fraaije*

ACS Synth. Biol. 2024, 13, 921–929

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ABSTRACT Industrial application of the natural deazaflavin cofactor F₄₂₂ has high potential for the enzymatic synthesis of high value compounds. It can offer an additional range of chemistry to the use of well-explored redox cofactors such as FMN and their respective enzymes. Its limited access through organisms that are either difficult to grow has urged research on the heterologous production of F₄₂₂ using more industrially relevant microorganisms such as *Escherichia coli*. In this study, we demonstrate the possibility of producing this cofactor in a robust and widely used industrial organism, *Saccharomyces cerevisiae*, by the heterologous expression of the F₄₂₂ pathway. Through careful selection of involved enzymes and some optimization, we achieved an F₄₂₂ yield of ~0.3 pmol/L, which is comparable to the yield of natural F₄₂₂ producers. Furthermore, we showed the potential use of F₄₂₂-producing *S. cerevisiae* for F₄₂₂-dependent bioconversions by carrying out the whole-cell conversion of tetraizole. As the first demonstration of F₄₂₂ synthesis and use for bioconversion in a eukaryotic organism, this study contributes to the development



KEYWORDS F₄₂₂, F₄₂₂ biosynthesis, *S. cerevisiae*, tetraizole bioisynthesis, F₄₂₂-dependent bioconversion

INTRODUCTION
F₄₂₂ is a naturally occurring deazaflavin cofactor synthesized only by certain bacteria and archaea, such as *Acetivibrio* and *Halobacterium* species. While having a similar structure to the ubiquitous flavin cofactor FMN, the chemical properties of F₄₂₂ are more like nicotinamide cofactors, as it exclusively performs hydride transfer reactions due to the CS of the 5-deaza-isoalloxazine moiety. F₄₂₂-dependent reactions catalyze the asymmetric reduction of imines, ketones, enones, etc., and can potentially be used as an alternative to flavin-containing and other NAD(P)H-dependent reductions.^{1–4} Furthermore, the low redox potential of F₄₂₂ compared to the flavin cofactors FMN and FMAD, and even to NAD(P)H, allow the reduction of recalcitrant substrates, expanding the scope of the currently available applications of enzymatic reductions.⁵

Despite the potential use of F₄₂₂ for various industrial applications, the biosynthesis of this cofactor is limited to the use of natural producers such as *Halobacterium* organisms, which hinder the cofactor availability and thus the related research. Therefore, the heterologous production in more versatile organisms such as *Escherichia coli* and yeast can be an attractive solution for easy access to this deazaflavin cofactor. Previous studies have shown that yeast is able to produce F₄₂₂ and analogues in *E. coli* by heterologous expression of the F₄₂₂ biosynthetic pathway, demonstrating the potential use of this organism for the cofactor production, as well as F₄₂₂-dependent

bioconversion.^{6–9} As a substitute to F₄₂₂, a synthesis of structurally much simpler and yet functional non-natural deazaflavin analogue FOP has also been explored using both *E. coli* and *Saccharomyces cerevisiae*, offering an attractive alternative solution.¹⁰

Either naturally or non-naturally, F₄₂₂ has so far been synthesized only in prokaryotic organisms. In this study, we explored the biosynthesis of F₄₂₂ in *S. cerevisiae* to extend the F₄₂₂-dependent biosynthesis platform even further to eukaryotic organisms. *S. cerevisiae* is a widely used organism across laboratories and industries due to its robust and harmless nature as well as its well-understood biological properties and well-developed molecular biological tools. Therefore, producing F₄₂₂ in this versatile organism can expand biotechnological means for related research and applications.

The biosynthetic pathway of F₄₂₂ (Figure 1) is well characterized and information on the chemical and structural properties of the involved enzymes from a few representative organisms such as *M. smitensis* and *Halobacterium* are available.

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RESEARCH ARTICLE | Yeast WILEY

Implication of polymerase recycling for nascent transcript quantification by live cell imaging

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Abstract
Transcription enables the production of RNA from a DNA template. Due to the highly dynamic nature of transcription, live-cell imaging methods play a crucial role in measuring the kinetics of this process. For instance, transcriptional bursts have been visualized using fluorescent phase-coat proteins that associate tightly with messenger RNA (mRNA) stem loops formed on nascent transcripts. To convert the signal emanating from a transcription site into meaningful estimates of transcription dynamics, the influence of various parameters on the measured signal must be evaluated. Here, the effect of gene length on the intensity of the transcription site focus was analyzed. Intriguingly, a longer gene can support a larger number of transcribing polymerases, thus leading to an increase in the measured signal. However, measurements of transcription induced by hyper-osmotic stress responsive promoters display independence from gene length. A mathematical model of the stress-induced transcription process suggests that the formation of gene loops that favor the recycling of polymerase from the terminator to the promoter can explain the observed behavior. Our experimentally validated prediction from the model is that the amount of mRNA produced from a short gene should be higher than for a long one as the density of active polymerase on the short gene will be increased by polymerase recycling. Our data suggest that this recycling contributes significantly to the promoter output from a gene and that polymerase recycling is modulated by the promoter identity and the cellular state.

KEYWORDS gene looping, MAPK signaling pathways, phase-coat proteins, stress response, transcription dynamics

1 | INTRODUCTION
Messenger RNA (mRNA) molecules are essential intermediates between acting cellular proteins and information encoded in DNA. The production of mRNA is an intricate process that involves the interaction between numerous complexes to initiate transcription, elongate the transcript and finally produce a matured mRNA molecule (Hahn & Young, 2011; Shanley & Roberts, 2012). Biochemical analyses have allowed characterization of the various complexes implicated in the production of mRNA (de Nadal

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RESEARCH ARTICLE | Traffic WILEY

Mechanisms regulating the intracellular trafficking and release of CLN5 and CTSD

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Abstract
Ceroid lipofuscinosis neuronal 5 (CLN5) and cathepsin D (CTSD) are soluble lysosomal enzymes that also localize extracellularly. In humans, homozygous

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CELLULAR, A Cell Autophagy Imaging Dataset

Amari Al Otaibi^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103,104,105,106,107,108,109,110,111,112,113,114,115,116,117,118,119,120,121,122,123,124,125,126,127,128,129,130,131,132,133,134,135,136,137,138,139,140,141,142,143,144,145,146,147,148,149,150,151,152,153,154,155,156,157,158,159,160,161,162,163,164,165,166,167,168,169,170,171,172,173,174,175,176,177,178,179,180,181,182,183,184,185,186,187,188,189,190,191,192,193,194,195,196,197,198,199,200,201,202,203,204,205,206,207,208,209,210,211,212,213,214,215,216,217,218,219,220,221,222,223,224,225,226,227,228,229,230,231,232,233,234,235,236,237,238,239,240,241,242,243,244,245,246,247,248,249,250,251,252,253,254,255,256,257,258,259,260,261,262,263,264,265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280,281,282,283,284,285,286,287,288,289,290,291,292,293,294,295,296,297,298,299,300,301,302,303,304,305,306,307,308,309,310,311,312,313,314,315,316,317,318,319,320,321,322,323,324,325,326,327,328,329,330,331,332,333,334,335,336,337,338,339,340,341,342,343,344,345,346,347,348,349,350,351,352,353,354,355,356,357,358,359,360,361,362,363,364,365,366,367,368,369,370,371,372,373,374,375,376,377,378,379,380,381,382,383,384,385,386,387,388,389,390,391,392,393,394,395,396,397,398,399,400,401,402,403,404,405,406,407,408,409,410,411,412,413,414,415,416,417,418,419,420,421,422,423,424,425,426,427,428,429,430,431,432,433,434,435,436,437,438,439,440,441,442,443,444,445,446,447,448,449,450,451,452,453,454,455,456,457,458,459,460,461,462,463,464,465,466,467,468,469,470,471,472,473,474,475,476,477,478,479,480,481,482,483,484,485,486,487,488,489,490,491,492,493,494,495,496,497,498,499,500,501,502,503,504,505,506,507,508,509,510,511,512,513,514,515,516,517,518,519,520,521,522,523,524,525,526,527,528,529,530,531,532,533,534,535,536,537,538,539,540,541,542,543,544,545,546,547,548,549,550,551,552,553,554,555,556,557,558,559,560,561,562,563,564,565,566,567,568,569,570,571,572,573,574,575,576,577,578,579,580,581,582,583,584,585,586,587,588,589,590,591,592,593,594,595,596,597,598,599,600,601,602,603,604,605,606,607,608,609,610,611,612,613,614,615,616,617,618,619,620,621,622,623,624,625,626,627,628,629,630,631,632,633,634,635,636,637,638,639,640,641,642,643,644,645,646,647,648,649,650,651,652,653,654,655,656,657,658,659,660,661,662,663,664,665,666,667,668,669,670,671,672,673,674,675,676,677,678,679,680,681,682,683,684,685,686,687,688,689,690,691,692,693,694,695,696,697,698,699,700,701,702,703,704,705,706,707,708,709,710,711,712,713,714,715,716,717,718,719,720,721,722,723,724,725,726,727,728,729,730,731,732,733,734,735,736,737,738,739,740,741,742,743,744,745,746,747,748,749,750,751,752,753,754,755,756,757,758,759,760,761,762,763,764,765,766,767,768,769,770,771,772,773,774,775,776,777,778,779,780,781,782,783,784,785,786,787,788,789,790,791,792,793,794,795,796,797,798,799,800,801,802,803,804,805,806,807,808,809,810,811,812,813,814,815,816,817,818,819,820,821,822,823,824,825,826,827,828,829,830,831,832,833,834,835,836,837,838,839,840,841,842,843,844,845,846,847,848,849,850,851,852,853,854,855,856,857,858,859,860,861,862,863,864,865,866,867,868,869,870,871,872,873,874,875,876,877,878,879,880,881,882,883,884,885,886,887,888,889,890,891,892,893,894,895,896,897,898,899,900,901,902,903,904,905,906,907,908,909,910,911,912,913,914,915,916,917,918,919,920,921,922,923,924,925,926,927,928,929,930,931,932,933,934,935,936,937,938,939,940,941,942,943,944,945,946,947,948,949,950,951,952,953,954,955,956,957,958,959,960,961,962,963,964,965,966,967,968,969,970,971,972,973,974,975,976,977,978,979,980,981,982,983,984,985,986,987,988,989,990,991,992,993,994,995,996,997,998,999,1000,1001,1002,1003,1004,1005,1006,1007,1008,1009,1010,1011,1012,1013,1014,1015,1016,1017,1018,1019,1020,1021,1022,1023,1024,1025,1026,1027,1028,1029,1030,1031,1032,1033,1034,1035,1036,1037,1038,1039,1040,1041,1042,1043,1044,1045,1046,1047,1048,1049,1050,1051,1052,1053,1054,1055,1056,1057,1058,1059,1060,1061,1062,1063,1064,1065,1066,1067,1068,1069,1070,1071,1072,1073,1074,1075,1076,1077,1078,1079,1080,1081,1082,1083,1084,1085,1086,1087,1088,1089,1090,1091,1092,1093,1094,1095,1096,1097,1098,1099,1100,1101,1102,1103,1104,1105,1106,1107,1108,1109,1110,1111,1112,1113,1114,1115,1116,1117,1118,1119,1120,1121,1122,1123,1124,1125,1126,1127,1128,1129,1130,1131,1132,1133,1134,1135,1136,1137,1138,1139,1140,1141,1142,1143,1144,1145,1146,1147,1148,1149,1150,1151,1152,1153,1154,1155,1156,1157,1158,1159,1160,1161,1162,1163,1164,1165,1166,1167,1168,1169,1170,1171,1172,1173,1174,1175,1176,1177,1178,1179,1180,1181,1182,1183,1184,1185,1186,1187,1188,1189,1190,1191,1192,1193,1194,1195,1196,1197,1198,1199,1200,1201,1202,1203,1204,1205,1206,1207,1208,1209,1210,1211,1212,1213,1214,1215,1216,1217,1218,1219,1220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