**Supplementary Materials**

**to Shalaeva *et al*. “Evolution of cation binding in the active sites of P-loop nucleoside triphosphatases”**

**Supplementary file 1A. Monovalent cation requirements of P-loop GTPases and ATPases**

|  |  |  |  |
| --- | --- | --- | --- |
| **TRAFAC class** | | | |
| **Protein name** | **UniProt ID** | **Cation dependence** | **Reference** |
| Dynamin-1 | DYN1\_HUMAN | K+>Na+ | (1) |
| Dynamin-related protein 1A | DRP1A\_ARATH | K+, Na+ | (2) |
| GTPase Nug1 | G0SEW3\_CHATD | K+>Na+ | (3) |
| Ribosome biogenesis GTPase A | RBGA\_BACSU | K+, no Na+ | (4) |
| Ribosome biogenesis GTPase RsgA (YjeQ) | RSGA\_ECOLI | K+ | (5) |
| Elongation Factor Tu, *E. coli* | EFTU1\_ECOLI | K+>Na+ | (6) |
| Elongation Factor Tu, *Haloarcula marismortui* | EF1A\_HALMA | K+>Na+ | (7) |
| Eukaryotic translation initiation factor 5B | IF2P\_CHATD | Na+, K+ | (8) |
| Initiation factor IF-2 | IF2\_ECOLI | K+ | (9) |
| tRNA modification GTPase MnmE | MNME\_ECOLI | K+, no Na+ | (10) |
| Ferrous iron transporter B | Q5M586\_STRT2 | K+, no Na+ | (11) |
| Ribosome-binding ATPase YchF | YCHF\_ECOLI | K+, no Na+ | (12) |
| GTPase HflX\* | HFLX\_BACSU | K+ | (13) |
| GTPase Era | ERA\_BACSU | K+, no Na+ | (13) |
| GTP-binding protein EngA\*\* *B. subtilis* | DER\_BACSU | K+, no Na+ | (13, 14) |
| GTP-binding protein EngA\*\* *T. maritima* | DER\_THEMA | K+, no Na+ | (15) |
| NO-associated protein 1 | NOA1\_ARATH | K+ | (16) |
| Ribosome Assembly GTPase YqeH | YQEH\_BACSU | K+, no Na+ | (17) |
| Developmentally-regulated GTP-binding protein 1 | DRG1\_HUMAN | K+ | (18) |
| GTP-binding protein EngB | ENGB\_BACSU | K+\* | (13) |
| Human GTPBP3 | GTPB3\_HUMAN | K+ | (19) |

|  |  |  |  |
| --- | --- | --- | --- |
| **RecA-like family** | | | |
| Human meiotic recombinase Dmc1 | DMC1\_HUMAN | K+ | (20) |
| Human DNA repair protein RAD51 | RAD51\_HUMAN | K+ | (21) |
| K+, no Na+ | (22) |
| Yeast DNA repair protein RAD51 | RAD51\_YEAST | K+ | (23) |
| DNA repair protein RadA from *M. voltae* | RADA\_METVO | K+ | (24) |
| DNA repair protein RadA from *M. maripaludis* | RADA\_METMI | K+, no Na+ | (25) |

In the ‘Cation dependence’ column, ’K+’ indicates that only K+-dependence has been shown; ’K+, no Na+’ indicates activation by K+ ions and a lack of activation by Na+ ions; ’K+>Na+’ denotes more effective activation by K+ than by Na+ ions; ’K+, Na+’ and ’Na+, K+’ is used when both cations have similar effects, with the more effective one listed first.

\* The GTPase activity was measured at the same concentrations of KCl and NaCl of 200 mM, and for some proteins (the second GTPase domain of EngA, HflX, EngB, all from *B. subtilis*), the lack of activation by cations has been reported (13). However, higher concentrations of ions may be required for these proteins in the absence of their activating partners, as has been shown for the second GTPase domain of EngA (14).

\*\* This protein has two P-loop GTPase domains, activity measurements were reported for the whole protein.

**Supplementary file 1B. Properties of monovalent cations and their interaction with the Mg2+-ATP complex.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cation** | **Ionic radius (Å)*b*** | **Stimulation of transphospho­rylation, %, *a*** | **Binding to ATP in the absence of Mg2+ (log(KB), 25°C** | | | **Binding to Mg-ATP (log(KB)*f*** |
| Na+ | 1.02 | 28 | 1.31±0.03*c* | 1.989±0.007*d* | 1.93*e* | **2.76** |
| K+ | 1.38 | 64-73\* | 1.17±0.03*c* | 1.873±0.005*d* | 1.99*e* | **0.88** |
| NH4+ | 1.44 | 27 | N/A | | | **1.76** |

\* measured for different salts: 64% with KCl and 73% with K2SO4.

*a* –data from (26); stimulation of transphosphorylation by 100 mM M+ in the presence of 50 μM MnCl2.

*b* – data from (27)

*c* – data from (28)

*d –* data from (29)

*e –* data from (30)

*f* – calculated from MD simulations

**Supplementary file 1C. Molecular dynamics simulations performed in this work**

|  |  |  |  |
| --- | --- | --- | --- |
| **No.** | **System** | **Simulation time** | **Number of repetitions** |
| 1 | Mg-ATP | 167 ns | 3 |
| 2 | Mg-ATP, K+ | 167 ns | 3 |
| 3 | Mg-ATP, Na+ | 167 ns | 3 |
| 4 | Mg-ATP, NH4+ | 167 ns | 3 |
| 5 | Mg-ATP | 20 ns | 25 |
| 6 | Mg-ATP, K+ | 20 ns | 25 |
| 7 | Mg-ATP, Na+ | 20 ns | 25 |
| 8 | Mg-ATP, NH4+ | 20 ns | 25 |
| 9 | Mg-GTP | 20 ns | 20 |
| 10 | Mg-GTP, K+ | 20 ns | 20 |
| 11 | Mg-GTP, Na+ | 20 ns | 20 |
| 12 | Mg-GTP, NH4+ | 20 ns | 20 |
| 13 | Mg-ATP, K+, w. positional restraints \* | 10 ns | 2 |
| 13 | Mg-GTP-MnmE, inactive, no K-loop, 3GEI | 100 ns | 1 |
| 14 | Mg-GTP-MnmE, inactive, K-loop, no K+, 2GJ8W | 100 ns | 1 |
| 15 | Mg-GTP-MnmE, active dimer with K+, 2GJ8K | 100 ns | 1 |

\* simulations were performed with positional restrains applied to monovalent cations bound in the AG and BG sites for analysis of dihedral angles of the phosphate chainmonovalent cations bound in the AG and BG sites, see the main text for details.

**Supplementary file 1D. Values of dihedral angles of the phosphate chains of Mg-ATP in the presence of K+ ions.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Structure** | **Ψα-β** | **Ψβ-γ** | **Ψα-γ** |
| Mg-ATP-K+  (MD simulation) | -60±23° | -4±18° | -59±35° |
| Mg-ATP-2K+  (MD simulation) | +13±24° | -27±8° | +1±26° |

Dihedral angle is an angle between two planes that is defined by four atoms. Values of dihedral angles between phosphate groups were defined as follows: Ψα-β = ∠O2A-PA-PB-O2B; Ψβ-γ = ∠O1B-PB-PG-O1G; andΨα-γ = ∠O1A-PA-PG-O3G, see also Fig. 3C. During the analysis of MD simulation data, the average and standard deviation values for dihedral angles were obtained by fitting the angle distribution histograms with normal functions, using the function “fit” in MatLab software (The Mathworks, Inc.). All distributions were fitted with one-term Gaussian models, except for the Ψβ-γ angle in case of the Mg-ATP with two K+ bound; this distribution was fitted with a two-term Gaussian, and parameters are shown for the highest peak. Distribution histograms and fitted curves are shown in Figure 3C.

\* The rotation of α-phosphate is unrestricted and the corresponding dihedral angles can take any values between -180° and 180°.

**Supplementary file 1E. Lifetimes of the βγ-conformation of Mg-ATP complex during MD simulations.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cation** | **K+** | **Na+** | **NH4+** | **no M+** |
| Average lifetime (ns) | 9.49 | 10.59 | 11.04 | 9.45 |
| Standard deviation | 6.52 | 8.28 | 7.82 | 7.85 |
| Lifetime (ns) for each MD run (total run time, 20 ns) | 7.68 | 13.18 | 0.88 | 0.91 |
| 16.16 | 0.15 | 19.75 | 3.43 |
| 16.8 | 1.18 | 19.55 | 0.61 |
| 7.93 | 11.8 | 8.48 | 14.73 |
| 4.93 | 4.03 | 2.71 | **20** |
| 0.28 | **20** | 2.21 | 12.38 |
| 6.06 | **20** | 1.58 | **20** |
| 2.75 | 7.01 | 12.8 | 19.23 |
| 11.76 | 3.86 | 10.71 | 0.26 |
| 6.33 | 20 | 2.65 | 0.93 |
| 13.43 | 2.36 | **20** | **20** |
| 2.65 | 20 | 16.98 | 6.2 |
| 8.11 | **20** | 17.66 | 0.21 |
| 11.21 | 20 | 9.16 | 10.58 |
| 4.9 | **20** | **20** | 3.03 |
| 8.03 | 16.41 | 1.18 | 13.38 |
| 0.7 | 3.15 | 1.21 | 1.03 |
| 14.68 | 2.25 | 1.06 | 5.31 |
| **20** | 10.93 | 8.36 | 0.36 |
| **20** | 0.18 | **20** | 9.11 |
| 0.38 | 6.83 | 5.63 | **20** |
| 4.68 | **20** | **20** | 6.2 |
| 8.01 | 0.66 | 20 | 8.48 |
| **19.75** | 0.83 | 13.38 | 20 |
| **20** | 20 | 20 | 20 |
|  |  |  |  |  |

For each system, 25 independent 20-ns MD simulation runs were conducted, each starting with the Mg-ATP complex in the βγ conformation. Stability of the βγ conformation was tracked by measuring the distance from the Mg2+ ion to the nearest oxygen atom of α-phosphate, and the time periods during which the βγ conformation was retained were compared between different systems. The one-way ANOVA analysis did not reveal any significant dependence of the stability of the βγ-coordination on the monovalent cation present. For each monovalent cation, the βγ-coordination was retained during the whole 20 ns in at least four cases (shown by bold numbers). These simulations were used to characterize the shape of the phosphate chain of ATP with βγ-coordination of the Mg2+ ion (Table 1 and Figure 5B).

**Supplementary file 1F.** Characteristics of the triphosphate chain for different interactions between the Mg2+ ion and ATP.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| System | Conformation | Pα-Pγ distance, Å | Number of frames | Pβ-O-Pγ angle, ° | Number of frames |
| No cations | βγ conformation | 5.4±0.3 | 160 | 122.4±3.5 | 640 |
| K+ | βγ conformation | 4.9±0.2 | 85 | 128.4±3.5 | 388 |
| Na+ | βγ conformation | 4.8±0.1 | 109 | 128.2±3.5 | 473 |
| NH4+ | βγ conformation | 4.9±0.2 | 64 | 128.6±3.8 | 251 |
| No cations | αβγ conformation | 4.7±0.2 | 161 | 125.2±3.3 | 267 |
| K+ | αβγ conformation | 4.3±0.1 | 133 | 127.9±3.6 | 198 |
| Na+ | αβγ conformation | 4.2±0.1 | 129 | 127.9±3.8 | 192 |
| NH4+ | αβγ conformation | 4.2±0.1 | 129 | 128.1±3.7 | 190 |
| Na+ | “curled” conformation | 4.6±0.2 | 131 | 124.3±3.2 | 194 |
| NH4+ | “curled” conformation | 4.6±0.2 | 125 | 124.9±3.5 | 183 |

**Supplementary file 1G.** Comparison of the PA-PG distance measurements of the βγ-coordinated Mg-ATP complexes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| System  (number of frames) | No M+ ions | K+ | Na+ | NH4+ |
| No M+ ions  (160) | N/A | 10-25 | 10-46 | 10-24 |
| K+  (85) | 10-25 | N/A | 10-7 | 0.16\* |
| Na+  (109) | 10-46 | 10-7 | N/A | 0.0041 |
| NH4+  (64) | 10-24 | 0.16\* | 0.0041 | N/A |

The null hypothesis was that the PA-PG distances in the βγ-coordinated Mg-ATP systems with different M+ ions added result from normal distributions with equal mean values.

\* The null hypothesis is NOT rejected, no significant difference between samples

**Supplementary file 1H.** Comparison of the PA-PG distance measurements of the αβγ-coordinated Mg-ATP complexes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| System  (number of frames) | No M+ ions | K+ | Na+ | NH4+ |
| No M+ ions  (161) | N/A | 10-56 | 10-78 | 10-76 |
| K+  (133) | 10-56 | N/A | 10-18 | 10-11 |
| Na+  (129) | 10-78 | 10-18 | N/A | 10-4 |
| NH4+  (129) | 10-76 | 10-11 | 10-4 | N/A |

The null hypothesis was that the PA-PG distances in the αβγ-coordinated Mg-ATP systems with different cations added result from normal distributions with equal mean values.

**Supplementary file 1I.** Comparison of the PA-PG distance measurements for the αβγ-coordinated and “curled” βγ-coordinated Mg-ATP complexes in different systems

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| System and conformation  (number of frames) | Na+, αβγ | Na+, “curled” | NH4+, αβγ | NH4+, “curled” |
| Na+, αβγ  (129) | N/A | 10-46 | 10-4 | N/A |
| Na+, “curled”  (121) | 10-46 | N/A | N/A | 0.98\* |
| NH4+, αβγ  (129) | 10-4 | N/A | N/A | 10-42 |
| NH4+, “curled”  (135) | N/A | 0.98\* | 10-42 | N/A |

The null hypothesis was that the PA-PG distances in ATP are the same in the αβγ-coordinated and “curled” βγ-coordinated Mg-ATP complexes, respectively.

\* The null hypothesis is NOT rejected, no significant difference between samples

**Supplementary file 1J.** Comparison of the PB-O3B-PG angle measurements for the βγ-coordinated Mg-ATP complexes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| System  (number of frames) | No M+ ions | K+ | Na+ | NH4+ |
| No M+ ions  (640) | N/A | 10-118 | 10-127 | 10-94 |
| K+  (388) | 10-118 | N/A | 0.46\* | 0.49\* |
| Na+  (473) | 10-127 | 0.46\* | N/A | 0.17\* |
| NH4+  (251) | 10-94 | 0.49\* | 0.17\* | N/A |

The null hypothesis was that the PB-O3B-PG angles in the βγ-coordinated Mg-ATP complexes with different M+ ions added result from normal distributions with equal mean values.

\* The null hypothesis was NOT rejected, no significant difference between samples

**Supplementary file 1K.** Comparison of the PB-O3B-PG angle measurements for the αβγ-coordinated Mg-ATP complexes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| System  (number of frames) | No M+ ions | K+ | Na+ | NH4+ |
| No M+ ions  (267) | N/A | 10-16 | 10-15 | 10-17 |
| K+  (198) | 10-16 | N/A | 0.94\* | 0.72\* |
| Na+  (192) | 10-15 | 0.94\* | N/A | 0.68\* |
| NH4+  (190) | 10-17 | 0.72\* | 0.68\* | N/A |

The null hypothesis was that the PB-O3B-PG angles in the αβγ-coordinated Mg-ATP complexes with different M+ ions added result from normal distributions with equal mean values.

\* The null hypothesis was NOT rejected, no significant difference between samples

**Supplementary file 1L.** Comparison of the PA-PG distance measurements for the αβγ-coordinated and “curled” βγ-coordinated Mg-ATP complexes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| System  (number of frames) | Na+, αβγ | Na+, “curled” | NH4+, αβγ | NH4+, “curled” |
| Na+, αβγ  (192) | N/A | 10-22 | 0.68\* | N/A |
| Na+, “curled”  (194) | 10-22 | N/A | N/A | 0.045 |
| NH4+, αβγ  (190) | 0.68\* | N/A | N/A | 10-16 |
| NH4+, “curled”  (183) | N/A | 0.045 | 10-16 | N/A |

The null hypothesis was that PA-PG distances are similar for the αβγ-coordinated and βγ-coordinated, "curled" Mg-ATP complexes.

\* The null hypothesis was NOT rejected, no significant difference between samples

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