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# EFFECT OF MUTATIONS AND PHOSPHORYLATION ON PYRIN STRUCTURE

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Computer models of mutant and phosphorylated forms of pirin have been generated. A comparison of the structures showed differences in pyrin structure which can affect complex formation of pyrin with other effectors.

*FMF – pyrin – computer modeling*

Համակարգչային մոդելավորման եղանակով ստացվել են մուտանտ և ֆոսֆորիլացված պիրինի մոլեկուլի երրորդային կառուցվածքները: Ստացված մոդելների համեմատությունը ցույց է տալիս որոշակի տարբերություններ, որոնք կարող են խաթարել պիրինի և այլ էֆեկտորների փոխազդեցությունը:

*ՄԸՏ – պիրին – համակարգչային մոդելավորում*

Методами компьютерного моделирования получены трехмерные модели мутантных и фосфорилированных форм пирина. Их сравнение показало различия в структуре, которые могут влиять на комплексообразование с другими белками-эффекторами.

*ССЛ – пирин – компьютерное моделирование*

MEFV gene, mutations of which brings manifestation of Familial Mediterranean Fever (FMF), localized on the short arm of 16$^{th}$ chromosome (16p13.3), consists of 10 exons and encoded pyrin, a protein of 781 amino acids. Although FMF manifestation molecular mechanisms are not completely clear, there are many data supporting hypothesis, that pyrin plays a key role in the process. The protein is localized mainly in cytoplasm of several type of leukocytes. It functions via complex formation with several other proteins and factors, participating in the processes of inflammation and apoptosis. On the other hand, pyrin translocates into nucleoplasm, which depends on alternative splicing and complex formation, that could also be functionally important [12, 8]. Now it is clear, that cytoplasmic pyrin interacts with caspase-1, ACS, and there are also indirect evidences regarding possibility of interaction with 14-3-3, p65, IkB-α, etc. Proteins of 14-3-3 family have an ability to bind many signaling molecules, and investigation of pyrin- 14-3-3 could illuminate function of pyrin and mechanisms of FMF pathogenesis. Currently very little is known about this interaction, the only data

shows, that interaction is taking place with phosphorylated sites of pyrin, localized at 200-250 region. It is interesting to note, that in these sites several mutations, associated with FMF, are known [8].

Taking into account all abovementioned, the purpose of this study has been to detect how mutations S208A, S209A, S242A, S208-209A, S208-209-242A and phosphorylation could change secondary and tertiary structure of pyrin. Based on that, two objectives have been formulated:

• To generate models of secondary and tertiary structure of abovementioned mutated and phosphorylate forms of pyrin based on the proposed computer model of native pyrin

• To compare influence of mutations and phosphorylation of pyrin on the ability of complex formation with 14-3-3 and consequence of mutations on physiological response related to the inflammation and apoptosis.

***Materials and methods***. For modeling of tertiary and secondary structures of mutated and phosphorylated forms of pyrin [2] software ROSETTA version 3.5 has been used. Modeling according to homology method has been applied, using computer model of native pyrin as a template, as has been described earlier.

Visualization and comparative study of native and mutant proteins have been performed with the use of VMD, version 1.9.2 [7]. Abovementioned software was used in the Linux system on 24-core computer cluster of IMB NAS RA [6] and on supercomputer complex of MSU [1].

***Results and Discussion.*** Based on the structure of native pyrin computer model, 10000 possible models for each of five investigated mutations have been generated with the help of ROSETTA software, using homology computation approach. After that, the best model for each mutation has been selected, based on minimal Gibbs energy and highest occurrence among generated models. For visible demonstration of structural differences between native pyrin and S208A, S209A, S242A, S208-209A, S208-209-242A mutations, we generated linear secondary structures for each mutations and compare it with secondary structure of native pyrin, results of which are presented in fig.1.

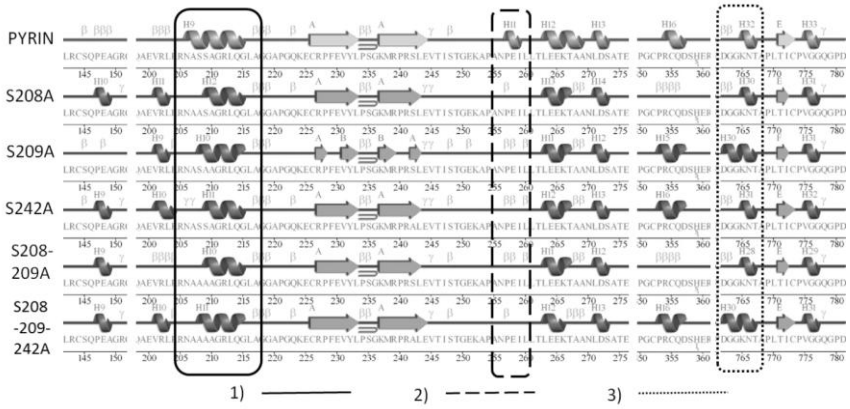


**Fig. 1.** Comparison of secondary structures of native and mutant forms of pyrin. Regions with changes are shown in the boxes. In the box 1, α-helix at the 206-215 position is shortening, in the box 2 α-helix at 207-209 is converting into a loop for all mutations, in the box 3, position at position 750-765 for mutations S209A and S208-209 elongation of α-helix is detected.

Since the region 750-765 is located in the B30.2 domain, we (believe), that some of these mutations cause structural changes not only in the sites, where mutations are located, but also affect other regions such as N-terminal part of pyrin. Generally, these

mutations lead to the creation of new α-helixes, their prolongation or shortening. Changes, located in the B30.2 region, seem very important from functional point of view, as the region is mainly responsible for genesis of the most severe mutations. In case of S209A mutation, located in the region of 227-244 AA, we detect formation of four β-sheets, instead of two in the native protein.

Since one of the purposes of our study was to check the possibilities of complex formation between pyrin and 14-3-3 and because we know, that this interaction is taking place only with phosphorylated sites of pyrin, we focused on the effect of mutations on pyrin's phosphorylation. In the native pyrin 208, 209 and 242 serines can be phosphorylated, which promotes the process of complex formation with 14-3-3. In the mutated forms phosphorylation was possible only in the sites, that were not affected by mutations (fig. 2).

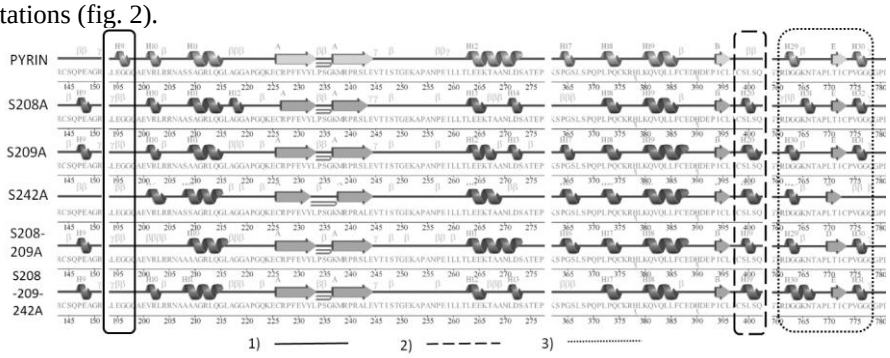


**Fig. 2.** Comparison of secondary structures of native and mutated forms of phosphorylated pyrin. Modifications, that are typical for all selected mutations, α-helix on the site 195-198, transformed into loop (1), the loop in the site 399-401 transformed into α-helix (2).

Phosphorylation, besides of structural changes, also dramatically change pyrin's ability to interact with 14-3-3 family [8]. It could be explained within the frames of the exchange of negatively charged serine into uncharged valine, which eliminates interaction with positively charged 14-3-3 domains.

There are certain similarities in the structures of investigated mutations. In the case of mutations located at 206-215 site, α-helix is shortened, and α-helix in the site 257-259 turns into a loop. In the case of comparison of the effect of mutations and phosphorylation a similar change is detected for α-helix in the site 195-198, which turns into a loop, and vice versa, the loop in the position 399-401 converts into α-helix in all mutated variants.

Abovementioned data are summarized in the table 1 (a, b)

**Table 1a.** Changes in the secondary structure of pyrin as the result of mutations in the 208, 209, 242 positions

| Mutation | 147-149 | 202-205 | 354-356 | 772-774 | 776-777 | 765-768 |
|---|---|---|---|---|---|---|
| Wild | loop | loop | α-helix | β-sheet | β-sheet | α-helix |
| S208A | α-helix | α-helix | loop | elongate | elongate | α-helix |
| S209A | loop | α-helix | loop | elongate | β-sheet | elongate |
| S242A | α-helix | loop | loop | β-sheet | β-sheet | α-helix |
| S208-209A | α-helix | α-helix | α-helix | elongate | elongate | α-helix |
| S208-209- 242A | α-helix | α-helix | loop | β-sheet | β-sheet | elongate |

**Table 1b.** Changes in the secondary structure of phosphorylated pyrin as the result of mutations in the same positions

| Mutation | 147-149 | 202-205 | 364-366 | 700-201 | 707-208 | 765-768 | 775-777 |
|---|---|---|---|---|---|---|---|
| Wild | loop | α-helix | α-helix | β-sheet | β-sheet | α-helix | α-helix |
| S208A | α-helix | α-helix | loop | elogate | elongate | α-helix | α-helix |
| S209A | α-helix | α-helix | α-helix | β-sheet | β-sheet | elongate | α-helix |
| S242A | loop | α-helix | α-helix | elogate | elongate | α-helix | loop |
| S208-209A | α-helix | loop | α-helix | elogate | elongate | α-helix | α-helix |
| S208-209-242A | α-helix | α-helix | loop | β-sheet | β-sheet | elongate | α-helix |

Summarizing abovementioned observations we can see, that mutations cause several structural changes, shown in the table, which are located not only around mutation site, but also in other remote regions; some of them can be very important in the triggering of FMF.

So, we can conclude, that mutations, localized in the 208, 209, 242 positions of pyrin molecule can influence not only local, but also global structure of pyrin, which in its turn could bring changes in complex formation process and change cell response triggering processes of inflammation, typical to FMF. Process of pyrin phosphorylation, which plays key role in pyrin 14-3-3 complex formation, is also sensitive to the point mutations of the region 200-250.

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