

INDUCTION OF A MAJOR STRESS PROTEIN SYNTHESIS IN MUSSELS MYTILUS EDULIS

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A major stress protein HSP70 synthesis in vivo and in vitro in a wheat-germ and rabbit-reticulocyte lysate cell-free systems using as a template poly(A)mRNA from mussels Mytilus edulis grown up in polluted crude oil hydrocarbons and relatively clear areas was investigated. It was concluded that inducible HSP70 synthesis in mussels from polluted areas may be as an adaptive reaction to the stress factors at the level of regulation of genetic activity.

Key words: major stress protein HSP70 synthesis, mussels *Mytilus edulis*.

Introduction. All organisms, pro- and eucaryotes, investigated to date respond to harmful environmental conditions by synthesis the so called stress proteins (Hartl et al., 2011). Induction of these proteins may be stimulated by adverse environmental conditions, such as variuos chemical pollutants, extreme temperatures, excessive salts, high-intensity irradiation and so on (Akerfelt et al., 2010). Some of stress proteins are synthesized constitutively under normal conditions. Functions of stress proteins seem to be varied. But it is clear now that they have a protective role in the organism during stress (Toshihide et al., 2015).

Mussels are well-known concentrators of chemical pollutants, and that's why may be a sensitive indicator system responding to actual marine pollution. Using this model the pollution-related changes of a magor stress protein synthesis were studied.

Materials and methods. Mussels of 3-6 cm length were transferred between the crude oil hydrocarbons polluted area, Kola Bay, near Murmansk, and the relatively clear area, aquarial plant in Dalnezelenetskaya Inlet. Proteins were labelled in vitro and in vivo during 30 min with L-[³⁵S]-methionine (1310 Ci per mmol "Amersham"). The proteins were extracted by homogenizing in tris-HCl buffered pH 7.5 solution (50 mM tris-HCl, pH 7.5; 100 mM NaCl; 2 mM DTT). Samples were centrifuged at 16000 g and the supernatants were analysed using 12.5% SDS-PAGE (King, Laemmli, 1971) and autoradiography and immunodot blot using the polyclonal *Drosophila melanogaster* HSP70-directed antibody. In vitro translation of poly(A)mRNAs isolated according to

Sala-Trepat et al. (1978) were performed in a wheat-germ (Roberts, Paterson, 1973) and rabbit-reticulocyte lysate (Safer et al., 1979) cell-free systems.

Results and discussion. Induction of synthesis of HSP70 in mussels *Mytilus edulis* of crude oil hydrocarbons polluted Kola Bay areas near Murmansk was observed. Level of HSP70 synthesis in mussels transferred from the polluted to relatively clear area (aquarial plant in Dalnezelenetskaya Inlet) over a period of 7-30 days was not decreased at all or decreased slightly. Probably there were great amount of crude oil hydrocarbons accumulated in mussels prior to their transference to aquarial plant that can't be eliminated from mussels during 7-30 days and so induce the HSP70 synthesis. However there were performed no investigation on crude oil hydrocarbons contents in mussels during their growth in relatively clear area in aquarial plant.

No HSP70 synthesis was observed in mussels grown up in Dalnezelenetskaya Inlet. Induction of HSP70 synthesis in the mussels was stimulated by their exposure to the water from Kola Bay (near Murmansk) polluted with crude oil hydrocarbons over one day. Synthesis of this protein stopped when mussels were returned to not polluted water.

However HSP70 are synthesized in vitro in a wheat-germ and rabbit-reticulocyte lysate cell-free systems using as a template poly(A)mRNA from mussels grown up in polluted and relatively clear areas. Existence of in vitro translatable mRNAs encoding HSP70 through the total poly(A)mRNAs from mussels grown up in clear water was confirmed by immunodot blot procedure. These results implies that genes encoding HSP70 are transcribed in normal conditions. So there is potential ability for constitutive in vivo synthesis of this protein in mussels in relatively clear area. But it is not clear at present why these mRNAs are not translated in normal conditions.

Conclusion. It may be assumed that inducible HSP70 synthesis in mussels *Mytilus edulis* in a polluted areas may be an adaptive reaction to the stress factors at the level of regulation of genetic activity. Probably this regulation is in part at the level of preexisting mRNAs for HSP70 translation.

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Анотація

Тихонов П.С. Індуція синтезу основного стресового білку мідій *Mytilus edulis*.

Досліджено синтез основного стресового білку HSP70 *in vivo* та *in vitro* у позаклітинних системах із зародків пшениці та лізату ретикулоцитів кролів з використанням як матриці полі(А)мРНК з мідій *Mytilus edulis*, що росли у забруднених вуглеводнями сирої нафти ареалах та у відносно чистих місцях. Дійшли висновку, що індукцйбельний синтез HSP70 у мідій із забруднених ареалів може бути адаптивною реакцією на стресові фактори на рівні регуляції генетичної активності.

Ключові слова: синтез основного стресового білку HSP70, мідій *Mytilus edulis*.

Аннотация

Тихонов П.С. Индукция синтеза основного стрессового белка мидий *Mytilus edulis*. Исследован синтез основного стрессового белка HSP70 *in vivo* и *in vitro* в бесклеточных системах из зародышей пшеницы и лизата ретикулоцитов кролика с использованием в качестве матрицы поли(А)мРНК из мидий *Mytilus edulis*, которые росли в загрязненных углеводами сырой нефти ареалах и в относительно чистых местах. Пришли к выводу, что индукцйбельный синтез HSP70 у мидий из

загрязненных ареалов может быть адаптивной реакцией на стрессовые факторы на уровне регуляции генетической активности.

Ключевые слова: *синтез основного стрессового белка HSP70, мидии *Mytilus edulis*.*