Induction of a 70,000 dalton protein in hypertrophic rat heart

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Summary. Two-dimensional gel electrophoresis analysis of the product of in vitro translation of polyadenylated RNAs extracted from rat heart rendered hypertrophic by aortic constriction, shows a new protein species not present in the map of control hearts. The same is also obtained when hypertrophy is induced by treatment with thyroxine.
Key words. Rat heart; hypertrophy; cell-free translation; two-dimensional electrophoresis.

Pressure overload by aortic constriction, or treatment with thyroid hormones, result in hypertrophic growth of adult rat heart. It has been clearly shown that this phenomenon is based on cell enlargement, rather than multiplication, and is supported, at the molecular level, by an increase in the rate of protein synthesis[1]. We have recently shown[2] that no qualitative changes occur in the one-dimensional gel electrophoresis pattern of the in vitro translation products encoded by purified messenger RNAs extracted from rat heart rendered hypertrophic by aortic constriction.

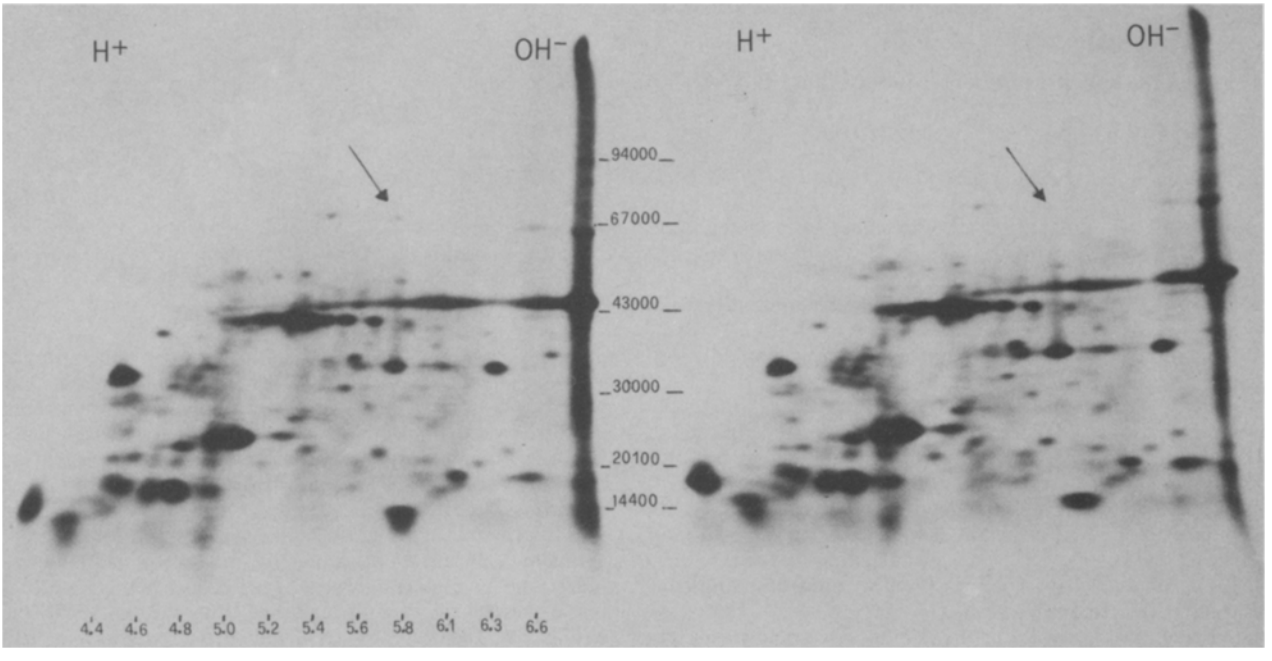
In the present paper we show that a novel protein species appears when the above translation products are resolved by means of two-dimensional gel electrophoresis. The same result is also obtained when heart hypertrophic growth is induced by treatment with thyroid hormone.

Materials and methods. Cardiac hypertrophy by abdominal aortic constriction was obtained in male Wistar rats weighing 200 g as previously described[2]. Ribonucleic acid (RNA) was extracted from fresh or frozen hearts by the guanidine hydrochloride procedure[3]; four hearts were pooled for each preparation. Polyadenylated RNA was selected by affinity chromatography on oligo dT cellulose (Type 7 from PL Biochemicals)[4]. Reticulocyte lysates were prepared and used essentially as described by Pelham and Jackson[5]. Typical reaction mixtures contained in 20 µl: 30 mM KCl, 10 mM Hepes pH 7.60, 0.5 mM ATP, 0.125 mM GTP, 0.3 mM spermidine, 4 mM creatine phosphate, 80 µM each of 19 amino acids (minus methionine), 40 µCi of [^{35}S] methionine (NEN 930–1260 Ci/mmol), 8 µl of nuclease-treated lysate, 1 µl of polyadenylated RNA (1 µg/µl).

Protein mixtures were resolved according to the two-dimensional electrophoretic procedure developed by O'Farrell[6], with minor modifications. Lyophilized samples were dissolved in 200 µl of lysis buffer: 9.5 M urea, 2% (w/v) Nonidet P40, 2% (w/v) Ampholines (LKB) in the following concentrations: pH 5.7 (1.6%) and pH 3.5–10 (0.4%). The samples were applied on cylindrical gels (3.5 × 10 mm) at the cathode and submitted to isoelectrofocusing: 300 V for 16 h, followed by 400 V for 4 h. The gels were then equilibrated for 15 min with 10 ml of equilibration buffer[6] and loaded onto a 8:20% (w/v) sodium dodecyl sulphate-polyacrylamide gradient gel.

Protein of known mol.wt (phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin-inhibitor, α-lactalbumin) running in the same gel as the sample were used for calculating mol.wts. After staining with Coomassie blue the gels were prepared for autoradiography as described by Bonner and Laskey[7] and exposed to Ilford X-ray film for 1–4 weeks.

Results and discussion. The present study was aimed at establishing whether specific proteins are produced in the rat heart undergoing hypertrophic growth as a result of different stimuli, such as aortic stenosis or administration of thyroxine. The proteins translated in vitro by messenger RNAs extracted from operated and control animals were resolved by two-dimensional gel electrophoresis (fig.). The pH gradient of the first dimension gel was



Two-dimensional gel electrophoresis of in vitro translation products directed by messenger RNA; 300,000 dpm were loaded for each sample onto the tube gels for isoelectrofocusing. The tubes were laid on the two sides of the same slab gel for the second dimension run. Right panel: normal animals. Left panel: hypertrophic animals after 7 days of aortic stenosis. This picture was obtained with messenger RNA extracted from 4 different pools of hearts.

4.3 at the anodal to 6.8 at the cathodal end. The mol.wt separation of the second dimension gel was from about 90,000 dalton at the top to about 10,000 dalton at the bottom. Under these conditions a new spot appeared consistently in the electrophoretograms of operated animals; by comparison of several in vitro translations this was shown to be an all-or-none response. The spot corresponds to a protein of an apparent mol.wt of 70 kdalton and an isoelectric pH of about 5.80. The general pattern of the in vitro translated proteins does not seem to be qualitatively influenced by the operation. Differences at the level of lower mol.wt proteins, such as those concerning the spot having the coordinate 14 kD-6.6 isolectric pH and 26 kD-6.0 isolectric pH, were only quantitative and not so reproducible. An identical map, including the new spot, was obtained when hypertrophic growth of the heart was induced by daily treatment with 1.0 mg/kg of thyroxine for 7 days (data not shown).

The new polypeptide induced by a stimulus causing hypertrophy does not appear to be produced in a large amount by the heart. In fact, it is not detectable in the map either of soluble or membrane-bound proteins extracted from hypertrophic and control hearts 7 days after operation. This was tested by direct analysis of the 100,000 × g × 2 h supernatant and of the pellet obtained thereafter, by two-dimensional gel electrophoresis followed by staining with Coomassie blue (data not shown). Similarly no differences could be found when the hearts were perfused in vitro (Langendorff apparatus)[8] for 60 min in the presence of [^{35}S] methionine and the protein extracted and analyzed by autoradiography, suggesting that under these conditions the protein is not produced at a sufficiently high rate to be visualized after methionine incorporation by this method.

Thus, hypertrophic growth of the rat heart resulting form aortic constriction or from treatment with thyroid hormone induces in vitro the synthesis of a protein species which is not obtained with hearts of normal animals. This may depend on specific gene derepression or specific activation of untranslated messenger RNA classes[9]. The new protein, which is not detectable before 7 days after operation or thyroxine treatment, seems to be a constant correlate of the hypertrophic growth of the heart, since it is induced by stimuli acting with completely different mechanisms. Apart from the 70 kdalton spot, the spectrum of in vitro translated heart proteins does not seem to change qualitatively after stenosis or thyroxine treatment. The large protein accumulation occurring during heart hypertrophic growth thus appears mostly to be sustained by an increased rate of production of the proteins normally synthesized within the heart. This can be based on a generalized increase in the rate of synthesis of all classes of messenger RNA[10] or/and on a more efficient utilization of the existing ones by means of activation of the translation process. We suggested previously[2] that this latter mechanism may be involved in rat heart enlargement by aortic stenosis, where we showed the activation of a factor that behaves like the eucaryotic initiation factor 2 (eIF-2).

Our further studies are directed toward establishing whether the new protein has a role in the development and maintenance of the hypertrophic state of the heart.

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Mating type-specific antibodies in the ciliate *Euplotes raikovi*[1]

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Summary. Three different types of gamones, each representing a different mating type of *Euplotes raikovi,* were used to elicit the production of antisera in mice. Each type of antiserum proved to be capable of preventing the gamone activity in correlation with the different specificities of the mating type.

Key words. Mating type; conjugation; gamone-antibodies; ciliates, *Euplotes.*

Conjugation in ciliates is a developmental process normally triggered by genetically controlled differences in the mating type products between conspecific cells[3]. These products are usually designated as mating type substances if they remain attached to the ciliary membranes, as in *Paramecium,* or as gamones if they are released into the extracellular environment, as in different species of *Blepharisma* and *Euplotes*[4]. Here we deal with gamones released by different mating types of *E. raikovi.* In practice, the gamone of cells of a given mating type diffuses into the environment where it can reach cells of another mating type and promote their conjugation.

Until recently the nature of the gamones was known only in *B. japonicum,* a species that has been considered as showing a dual mating type system[5]. The gamone of cells of mating type I is a glycoprotein of approximately 20 kD, whereas the gamone of cells of mating type II consists of a tryptophan derivative[5].

Gamones have now been identified in *E. raikovi* which, unlike *B. japonicum,* shows a high polymorphism of the locus for the mating type. For instance, seven different *mat* alleles have been identified by cross-breeding four of the 12 different mating types that were originally isolated after sampling a very restricted natural population[6,7]. The gamone released by cells of strain 13 was first isolated and characterized as a heat-stable glycoprotein of 12 kD, capable of promoting mate pair formation at a concentration of 0.036 ng/ml[8]. Subsequently, isolation of gamones was undertaken from other wild strains as well as from their descendant clones, which were distinguished from one another by the mating type. The new gamones characterized so far have proved to be glycoproteins similar in mol.wt and other significant chemical characteristics to the glycoprotein representing the gamone of strain 13[7].

To inquire into the nature of the different specificities within a