Regulation of immediate early gene expression by exercise: Short cuts for the adaptation of immune function

Running head: Immediate early genes in exercise immunology

Perikles Simon*, Elvira Fehrenbach and Andreas M. Niess***

*** Department of Sports Medicine, Medical Clinic and**
**** Institute for Clinical and Experimental Transfusion Medicine (IKET), University of Tübingen, Germany**

Abstract

Onset of physical activity induces a wide variety of rapid biophysical and biochemical responses that act on cells and lead to a wide range of phenotypic adaptations. Here we elucidate the role of immediate early gene (IEG) expression as a first line of transcriptional response that mediates protein neosynthesis and leads to adaptation of immune function. New posttranscriptional mechanisms have been described that speed up transcriptional responses. These include RNA-RNA interactions such as those exploited by miRNAs and stimulus-dependent cytoplasmic polyadenylation. We describe these shortcuts that modulate expression and discuss the challenges of accurately measuring them using various transcriptomic screening and quantification approaches.

Although there is high complexity of the upstream as well as the downstream pathways that lead to IEG expression, IEG expression itself may only show a limited number of response patterns. Focusing transcriptomic approaches in exercise immunology at the IEG-level may facilitate the discovery of exercise-specific transcriptional signatures. (Exerc. Immunol. Rev. 12, 2006: 112-131)

Key Words: Immediate early genes, posttranscriptional regulation, cytokines, ncRNA, cisNAT

1. Immediate early genes: The "sprinters" among our genes.

The term immediate early gene (IEG) was originally coined during the mid 1970s and early 1980s in reference to viral genes that were rapidly transcribed following invasion of a host (12;62;68). The main features of the IEG consist of a fast and

---

Address Correspondence to:
Perikles SIMON, MD, PhD, Department of Sports Medicine, Medical Clinic, University of Tübingen, Silcherstr. 5, D-72076 Tuebingen, Germany, Phone: ++49-7071-2985163, perikles@uni-tuebingen.de

transient transcriptional response after stimulation and several minutes well before the first expression of stimuli-related proteins. It has been shown that expression of IEGs is the key trigger leading to the initiation of protein neosynthesis and the subsequent expression of so-called"late response genes" that can only be activated following the translation of the small cohort of IEG transcripts (78).

With the discovery of the human homologs to retroviral IEG oncogenes the concept of IEGs was transferred to humans (15;50;75). In the beginning most of the IEGs, for instance the immediate early growth response gene 1 (Egr1), were characterized as transcription factors and key regulators of cell growth and differentiation (70). During the past 20 years it has been shown that 1. IEG function is not limited to transcription factor activity, 2. adaptation to a variety of stimuli including cytokines, mechanical stress, and somatosensory perception requires IEG expression, and 3. the subset of IEGs activated by a certain stimulus can be specific to a cell type and condition.

Here we propose a central role for IEGs in the adaptation of the immune system to physical stress and exercise. Transcriptomic approaches and improvements in RNA quantification have greatly facilitated the systematic analysis of IEG expression (25). Here we review results of some of the first studies using such approaches to study effects of physical exercise on expression in immune competent cells. We then describe how IEGs may exploit posttranscriptional processes such as cytoplasmic polyadenylation, degradation by Adenosine- and Uracil-rich element binding factors, and even expression of related non-coding RNAs (ncRNAs) to further accelerate their tightly controlled expression. These atypical mechanisms affect and perturb conventional mRNA expression assays.

2. The activation of immediate early genes in immune competent cells by physical exercise and stress

The majority of studies of IEG function have involved the central nervous system and rapid responses to activity and other environmental and experimental stimuli. Among roughly 180 reviews dealing with the term"immediate early gene/s" during the past 20 years more than 90 % are related to CNS function.

This is due to the fact that neuronal responses to stimuli – neuronal plasticity and learning – are processes physiologists agree depend on rapid molecular adaptations (54). However, there is nothing as crucial for the viability as the fast and robust responses to physical stress and exercise. Speed matters, not only for the immediate action itself, but also for the processes of rapid adaptations that inevitably require protein neosynthesis.

Immediate and effective upstream (earlier and causal) events have been identified which are known to induce effects in the various cell types of peripheral blood in response to exercise. Here we discuss the most prominent of these upstream events regarding their ability to induce IEG expression.

Upstream events can be subdivided into biophysical and biochemical events (see Fig. 1 for illustration). The former may principally act directly on peripheral blood cells or indirectly via induction of biochemical pathways anywhere within

the body. Accordingly, it is difficult to determine the source of many important immune modulators altered by exercise. This situation has already led to controversies in exercise physiology, as for instance in the case of the source for the increase in plasma IL6. The current opinion is now that it is rather the muscle cells than leukocytes which contribute to this effect (51, 67). This discussion can also be extended to alterations of any other kind of cytokines, including the extracellular heat shock proteins (eHSPs) with known cytokine function (3, 43). Clarification of the respective situation *in vivo* is not trivial given that just any organ or cell type, or even certain cell types neighbouring muscle cells, such as adipocytes or endothelial cells, could outrange production by peripheral blood cells or the muscle cells depending on type, duration and intensity of exercise. In terms of the question of IEG activation in leukocytes we can treat this complex of still to be addressed questions as a black box and look at the stimuli acting on leukocytes regardless of their origin.

Both biophysical and biochemical activation of cells rapidly elicits transcription of IEGs (38, 8, 74, 32). The upper part of Figure 1 gives an overview about biophysical and biochemical stimuli acting on cells following exercise. Despite the very diverse nature and a high interactivity of these stimuli, a striking observation is that they all activate MAP kinase cascades (8, 65, 18, 79, see Fig.1 middle). Induction of IEGs in a specific way is achieved, since the exact extent and time-course of activation of each of the ERK, JNK/SAPK and p38 MAP kinase cascades by a particular stimulus is highly distinctive and characteristic for that stimulus (see 7, 32, and references therein).

Events upstream of IEG expression in Leukocytes

Muscular activity is always combined with at least an increase of intramuscular temperature or even whole body temperature, which in turn activates expression of heat shock proteins (HSPs) (19, 23). Expression of HSPs is rapidly induced in an IEG fashion by the nuclear translocation of the heat shock transcription factor 1 upon posttranslational modification (60). The various functional aspects of HSPs on and in leukocytes include the protection from free radical induced DNA damage, induction of proinflamatory cytokine release, the direct activation of NK cells and a more general stimulation of adaptive, as well as innate immune responses. It is well known that these effects depend on intra- / extra-cellular localization, peptide loading status, origin and route of application (61).

HSPA1A (also termed HSP70, HSP70-1, HSP72, HSPA1 with alias) is known to be an IEG that is induced by heat and also by ischemia as another biophysical factor associated with exercise (13, 48). Elevated levels of extracellular HSPA1A, for instance, are found in plasma directly following exercise (21, 45). It has been implicated *in vivo* and *in vitro* that the ability of HSPA1A to activate the innate immune response by the release of NO and cytokines seemed to be improved in trained animals (6, 27). Interestingly, it has been shown that HSPA1A cooperates with heat shock factor 1 to repress expression of the immediate early gene c-fos; a central transcription factor for cell growth and differentiation (33). Repression of certain IEGs combined with activation of specific MAP-kinases therefore contributes to a stimuli specific IEG expression signa-

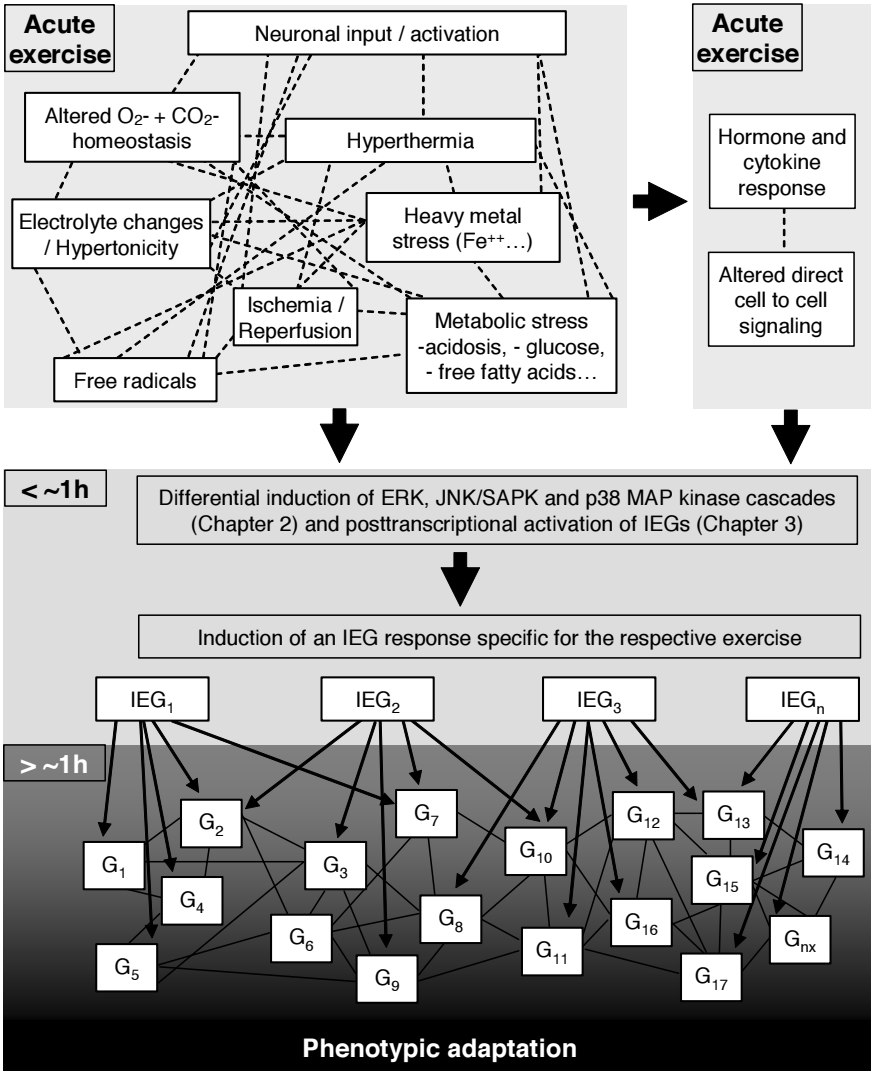


Fig.1: The upper part illustrates the main events upstream of the induction of IEG expression following exercise. Biophysical stimuli along with neuronal activation build up a complex interactive network (upper left) that generates a humoral response (upper right). The complex upper part leads to an induction of an IEG response specific for the respective exercise as described in chapters 2 and 3. The IEG response typically initialized 5min – 1h following exercise leads to the secondary induction of a more and more complex network of gene expression changes (G1-nx as arbitrary genes). As explained at the end of chapter 2, measuring gene expression at the level of the IEGs should enable us to get a limited number of gene expression signatures for a whole variety of different exercise regimes.

ture. As a consequence the complex transcriptional responses and the following protein neosynthesis are well adjusted to the intensity, duration and type of exercise (Fig.1 lower part).

Adaptation to increased temperatures *in vivo* is not readily distinguishable from the adaptation to mechanical forces that act directly on leukocytes, muscle cells, and endothelial cells during exercise. The process of mechanoperception is rather complex, since stretch-activated ion channels, caveolae, integrins, cadherins, growth factor receptors, myosin motors, cytoskeletal filaments, nuclei, extracellular matrix, and numerous other structures and signalling molecules have all been shown to contribute to the mechanotransduction response (37). Accordingly, there is a wide variety of IEGs that respond to mechanical force. Well characterized are for instance the induction of the IEGs c-fos, c-jun and PU1 in monocytes / macrophages (85) and the induction of Egr1 in endothelial cells (84). Especially the induction of Egr1 and c-fos is described for many different cell types and known to occur as quickly as 10 min following the stimulus (69).

Mechanical forces, in combination with heat and ischemia / reperfusion induce further biophysical stimuli, such as hyperosmosis and heavy metal stress (Fe^{++}). All of these changes are known to induce IEG expression via MAP-kinase signalling (63, 30). Hyperosmosis / hypertonicity induce expression of a variety of IEGs that carry a tonicity / osmotic response element (TonE / ORE) in their promoter region that is activated via MAP-kinase signalling (41, 77). HSPA1A is among the candidates that possess functional TonEs / OREs, along with other HSPs (34).

Another important biophysical stimulus related with exercise is the generation of free radicals (reviewed in 22 and 55). Free radicals activate the consensus sequence CC(A/T)6GG, known as a CArG box within the promoters of IEGs via serum response factor (16). Egr1 is a typical IEG well known to be activated through this pathway (16). Just recently, it has been shown that many genes of the immunoglobulin family have CArG-box elements, which provides an indirect link between free radicals and B-cell function (2).

In addition to the biophysical paths, there is a multitude of immediate hormonal and cytokine responses (reviewed in 71, 28, 72) as well as substrate and ion changes observed in the blood that add an additional level of complexity to the regulation of IEGs in leukocytes in response to exercise. The pathways leading to IEG expression again involve MAP-kinases and their action on respective enhancers and suppressors in the promoter regions of IEGs (53).

It should be pointed out that secretion of hormones and cytokines due to exercise can principally act as fast or even faster on leukocyte IEG expression than the biophysical stimuli itself. For both routes of IEG induction, there is a feature additional to MAP-kinase activation which is necessary for successful activation of an IEG. This is chromosomal remodelling necessary for the initiation of transcription (66, 73, 80). There is clear evidence of enhanced histone H3 and H4 acetylation, both at the promoters and within the body of genes upon transcriptional activation of IEGs (66).

If we summarize the upstream site to IEG expression in leukocytes due to physical activity, the following conclusions can be drawn (see Fig. 1 for illustration).

1. Many different biophysical and biochemical stimuli act together in order to induce IEG expression.
2. The initial stimuli acting on leukocytes to generate IEG expression are known to vary extensively depending on type, intensity and duration of exercise.
3. All stimuli act through the pathways of MAP-kinase activation in order to exhibit IEG expression.
4. Activation IEGs can be limited by a) the activation of other IEGs with direct or indirect repressor function as it is shown for HSPA1A and b) by the absence of a nucleosomal complex.
5. Every single IEG, once expressed, controls the expression of a variety of related late response genes.

Complexity of IEG response is limited by point 3 and 4. This leads to the situation that even though the upstream site to IEG expression, as indicated by points 1 and 2 and the downstream site (point 5), are rather complex there should be a much more simplified response on the IEG level (Fig.1). Various forms of physical exercise may all lead to a very limited number of different expression profiles on the IEG level. If this was the case, looking at IEG expression profiles following exercise would help us to categorize the different exercise regimes with regard to their effect on immune function.

First data about IEG expression in Leukocytes following exercise

Since many different biophysical and biochemical stimuli act together in order to induce IEG expression, data obtained by studying leukocyte expression following certain stimuli in vitro may be of very limited value for the field of exercise physiology. In addition, contact with foreign matter is a severe stimulus to leukocytes limiting the significance of in vitro studies. Approaches on the transcriptome level have already studied this effect (36).

The advantages of screening approaches on the transcriptome level for studying the effects of exercise on leukocytes *in vivo* have already been described and first results have been published (14, 25, 90). Comparison and interpretation of the results of such screening attempts may enable the deduction of specific transcriptomic signatures associated with certain exercise regimes (91).

Connolly et al. used Affymetrix HU133A GeneChips and investigated the expression differences in peripheral blood derived mononuclear cells (PBMCs) directly after and 1hour after a 30 min run at 80 % of the participants predefined VO_2max (14). This setting is particularly helpful to study IEG expression for two reasons. First, 30 min of exercise just enables the expression of IEGs and avoids differential expression of late response genes (see also Fig.1). Second, a typical IEG response is transient and would therefore rather be detected 30 min following the onset of exercise but not necessarily 1 hour after the end of exercise anymore. Therefore, this point in time helps to further categorize the IEG response found directly after exercise into a typical transient IEG expression and an atypical prolonged IEG expression. It has to be considered that the points in time chosen by

Connolly et al. are particularly suited for endurance exercise. More intense exercises and also weight lifting may require even shorter measuring intervals, since it is well known that many important IEGs show prominent gene expression changes as early as 10 min following the stimulus. In the case of endurance exercise, however, biophysical stimuli - such as heat – may take some time to reach a critical threshold to induce IEG expression. Since this delay might be specific for one or another biophysical or humoral stimulus, this situation may offer a chance to unravel the effect of certain stimuli on gene expression *in vivo*. Prerequisite is of course that studies are conducted with measuring intervals adjusted in this respect.

In the study by Connolly et al., Affymetrix HU133A GeneChips were used. Multiple single probe measurements are summarized on these chips to calculate a representative expression of a so called "probe set" that belongs to one mRNA. Quite often, more than one probe set belongs to one reference mRNA of a gene. 433 probe sets belonging to 337 different reference RNAs showed significant gene expression changes directly or 1 hour following exercise in the study of Connolly at all. Among these candidates 66 probe sets representing 55 different reference RNAs were altered more than 2-fold directly after 30 min of exercise (for > 3-fold change: 15 or 12 candidates respectively). All of these candidates showed a reduced level of expression 1 hour later and accordingly would meet the criteria of typical exercise induced IEGs in PBMCs. Among these candidates is HSPA1B encoding for a protein variant of HSP70 and HSPA1A as the classical candidate for the protein HSP70. HSPA1B showed a 1.96-fold increase 30 min following exercise.

Important for our first impression about exercise induced gene expression changes is the observation that 1 hour after exercise only 15 candidates showed a significantly increased expression ratio compared to the baseline level, which is just a fourth of the number seen directly following exercise. Given that we would principally expect an even higher number of early and late response genes being induced as a consequence of IEG expression, this finding needs to be explained. One reason could be the bias of the expression changes due to blood cell shifts. Connolly et al. showed that the ratio of lymphocytes to monocytes was on average 25 % elevated and 40 % decreased compared to baseline directly after and 1 hour after exercise. Expression of particular candidates varies between the various cell types among the PBMCs from not expressed to highly expressed and often shows a difference of more than a factor of 100 between cell types (56). Therefore the above mentioned cell shifts could principally be the cause of any of the significant gene expression changes observed regardless of the height of the respective fold-change ratio (10, 31).

A second study that investigated gene expression changes in PBMCs following exercise in a systematic way was done by Fehrenbach and Zieker et al. (25, 90). This study used a cDNA array centred on inflammation and looked for expression changes directly after and 24 hours after a half marathon competition. It could be shown that expression analysis of cell type specific surface molecules reflects the observed individual cellular shifts in peripheral blood cells with high statistical significance. Accordingly, Zieker et al. could focus their evaluation on those candidates which showed differential regulation that could not be explained by the cell shifts found. Directly following exercise an upregulation of MAPKAP-

K2, L-selectin and IL1-ra was reported to occur and this agrees with previous observations in exercise physiology studies based on different methods (90). Novel findings were a down-regulation of CD81 and GSTM3, as well as an up-regulation of thioredoxin. Since the average running time during this study was more than 1.5 hours, one cannot conclude whether these candidates can be considered as IEGs or as early response genes transcriptionally activated by the first IEGs.

A very interesting IEG candidate is MAPKAP-K2. So far, MAPKAP-K2 has only been described to be elevated in muscle cells following exercise (86). The increase in MAPKAP-K2 in PBMCs following exercise is of particular importance since it has been shown that this induces rapid induction of the protein expression of many inflammatory genes by posttranscriptional mechanisms. These will be discussed in the following section (47, 83).

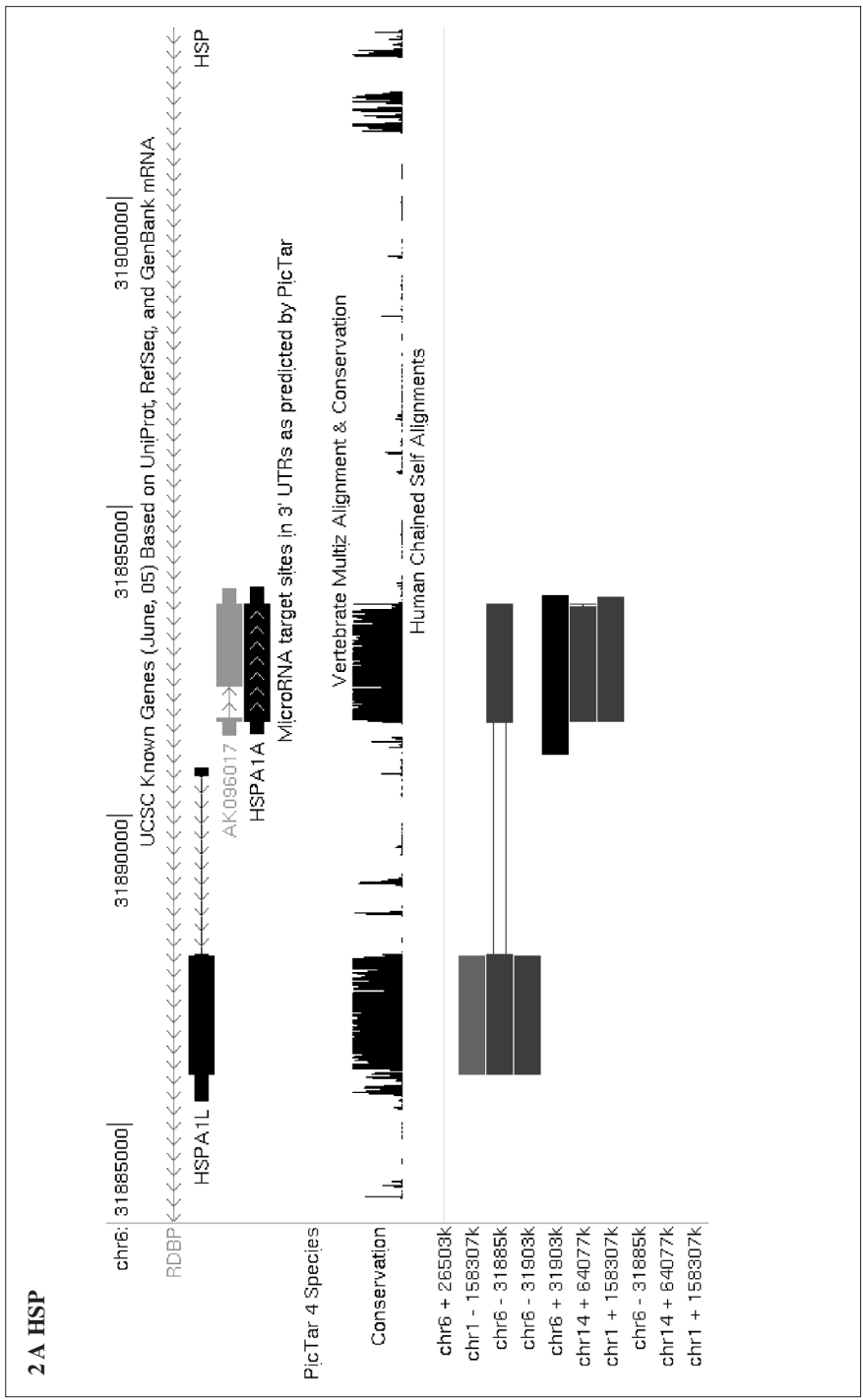
Future studies that aim at detecting IEG expression changes following exercise could combine the advantages of the two studies which used screening techniques so far. It would be informative to look at a timeframe between 30-40 min following onset of exercise and compare this to a second sampling point about 1-3 hours later. A classical IEG would show increased expression levels at the first point in time but already decreased values at the second point in time. It would also be important to control for the cell shifts occurring during the particular periods of exercise or recovery from exercise. Ideally one could integrate the just recently generated knowledge on the basal expression differences between the various cell types among the PBMCs in order to exclude that expression changes can be explained by cell shifts only (10, 31, 56).

3. New insights into the modulation of IEG expression: Pitfalls and potentials for future transcriptome oriented exercise immunology studies

The functional aspect of non-coding RNAs

This years Nobel prize in Physiology or Medicine was awarded for the discovery of RNA interference (RNAi) in 1998 (26). RNAi by the use of so-called micro RNAs (miRNAs) is one among many other mechanisms that act posttranscriptionally to control gene expression. miRNA belong to the class of non-coding RNAs (ncRNAs) that are not translated into proteins. Recently the magnitude of the expression of many different kinds of ncRNAs including miRNAs in the mammalian genome has been elucidated (9, 39). ncRNAs influence transcription, RNA editing including heterogeneous nuclear RNA (hnRNA) splicing, RNA transport, RNA stability, and RNA translation of many important protein coding genes (35, 52, 59, 81). Accordingly, ncRNAs can influence the expression level of many other coding RNAs and there is increasing evidence for their importance to modulate phenotypes (11, 35, 46, 76, 87).

Once, the intergenic spaces and introns were referred to as"genetic junk" and it is still true that only 1-2 percent of a mammalian genome contributes to exons of known genes that are expressed. However, there is evidence that ~60 % of the mouse genome is transcribed and that half of the roughly 181,000 independent transcripts represent ncRNAs (9, 39). 70 % of the mapped transcription


2 A HSP
chr6: 31885000
31890000
31895000
31900000
UCSC Known Genes (June, 05) Based on UniProt, RefSeq, and GenBank mRNA
RDBP
HSPA1L
AK096017
HSPA1A
HSP
MicroRNA target sites in 3' UTRs as predicted by PicTar
PicTar 4 Species
Vertebrate Multiz Alignment & Conservation
Conservation
Human Chained Self Alignments
chr6 + 26503k
chr1 - 158307k
chr6 - 31885k
chr6 - 31903k
chr6 + 31903k
chr14 + 64077k
chr1 + 158307k
chr6 - 31885k
chr14 + 64077k
chr1 + 158307k

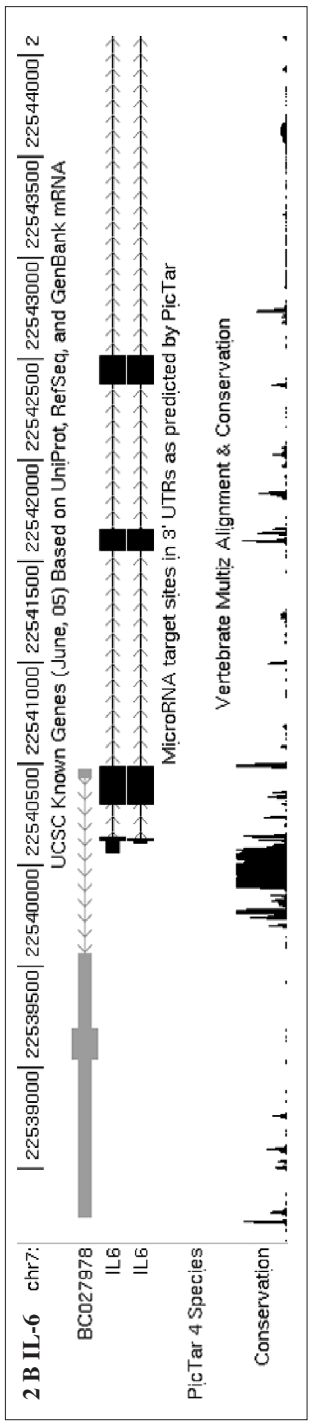


Fig. 2: The figure was generated by using the Genome Browser and its integrated bioinformatics software tools that are described in the following. It shows an alignment of four paralogs that code for HSPA1A (HSP70) protein variants to a locus on chromosome 6 (a) as well as an alignment of the two reference mRNA sequences to a locus on chromosome 7 (b). Lines represent introns, arrows are directed from 5' to 3' of the respective transcripts, thin boxes represent untranslated regions and thick boxes the coding sequence of a transcript. Target sites for miRNA were calculated using the PicTar algorithm and their localization is indicated as a thin line. In the"Conservation" diagram the maximum height of the line indicates complete phylogenetic conservation among 17 different vertebrate species. Below"Human Chained Self Alignments" the presence of the respective box indicates the existence of a complementary sequence to the respective box-locus at a different chromosomal location that is given on the left hand site. The darker the box, the higher is the sequence homology to the other chromosomal location. Further explanations are given in the text.

units seem to overlap to some extent with a transcript from the opposite strand (9, 39). This has led to the question "Transcription will never be simple again, but how complex will it get?"(11).

The complexity of the transcriptome is therefore another reason for concentrating on immediate early effects. It can be expected that the more time we wait following a stimulus the more complex the transcriptional response is going to appear (Fig. 1). In the next paragraphs we review how posttranscriptional regulation influences expression levels of IEGs.

Posttranscriptional regulation of IEG expression via recognition motives

An important issue influencing level of expression and detection of IEGs is the modification of RNA-stability and -translatability depending on stimulus-dependent posttranscriptional interactions at recognition motives located within an mRNA. Most of these recognition motives are located in the 3'- or to a less extent in the 5'-untranslated region (UTR) of an mRNA. Well characterized among these motives are cytoplasmic polyadenylation elements (CPEs) and Adenosin- and Uracil- rich elements (AREs). mRNAs that possess a CPE in their 3' UTR are polyadenylated in the

cytoplasm following stimulation, and the addition of the polyA tail leads to increased mRNA stability and translatability (5, 57). mRNAs with an ARE have been shown to be either stabilized or destabilized by binding of the protein HuR or AUF1 to the ARE, respectively (17, 58).

This situation is important for an understanding of gene expression regulation in exercise immunology, since many cytokines contain ARE elements in their 3' UTRs (4). The mRNAs of IL2, IL6, IL8, and TNFα all have been shown to be inherently unstable in unstimulated PBMCs due to the presence of AREs within their 3' UTRs. Following stimulation, IL6 and IL8 are stabilized by the activation of the MAPK-pathway. Therefore the MAPK-pathway is of central importance for the transcriptional, as well as post-transcriptional regulation of gene expression. Interestingly, MAPKAP-K2 has been shown to be effective to achieve the full stabilizing effect on IL6 and IL8 mRNA (83). Both cytokines are known to rise in the plasma following exercise (71). The discovery that MAPKAP-K2 is up-regulated in skeletal muscle and in PBMCs following exhaustive endurance exercise is therefore in line with a posttranscriptional regulation of IL6 and IL8 expression following exercise by MAPKAP-K2. In contrast to this situation the stabilization of IL2 and TNFα mRNA following T-cell activation is known to be independent of MAPKAP-K2 (82). Interestingly, these cytokine candidates are not reported to increase in plasma following exercise (71). Future studies may therefore elucidate the importance of MAPKAP-K2 expression and activity for mediating cytokine responses following exercise by taking posttranscriptional regulation of cytokines via AREs into account.

Posttranscriptional regulation and its impact on "detectable" IEG expression

Regulation of RNA expression via ncRNAs and recognition motives generally either stabilizes or destabilizes an mRNA that has already been transcribed. Both mechanisms are potential short cuts that modulate levels of a protein without requiring changes in basal transcriptional state. Mature and nearly mature mRNAs can be stored when and where they may be most needed for quick up-regulation of transcription.

In the following we will use information about posttranscriptional gene regulation and sequence alignment tools in order to illustrate the chances as well as the difficulties to detect differential regulation of IEGs by looking at HSPA1A and IL6 as an example.

Figure 2 illustrates prominent bioinformatic features of these two candidates that are well known to play a central role in exercise immunology. Concerning the phylogenetic conservation HSPA1A is a typical mRNA. Conservation across 17 species is very high for all the protein coding regions (thick boxes) ("Conservation" line in figure 2a), while the 5' UTR and 3' UTR (thin boxes) are not well conserved. IL6 shows the opposite pattern. Here the promoter region and especially the 3' UTR are highly conserved. How could it be that IL6 shows a much higher level of conservation in its UTRs than in the protein coding region, given that a high level of conservation implies functional relevance? One explanation is that the genetic code is redundant and therefore the IL6 proteins show a much higher level of conservation across species than

the respective coding sequences. In contrast, the recognition motives in UTRs have to be almost 100% conserved in order to maintain functionality across species.

Even though four HuR binding ARE-motives are located in the 3' UTR of IL6, the high level of conservation for almost the entire 500 bp 3' UTR can not be explained by the roughly 68–80 bp that are important for the four functional AREs (44). A more likely scenario is that there are additional recognition motives within this 3' UTR. The PicTar 4 Species track in Fig 2b uses a recently developed miRNA target site detection algorithm and highlights target sites for the miRNAs let-7, mir-26, mir-98, and mir-149 in the 3' UTR of IL6 (29, 42). Accordingly, targeted destruction of IL6 mRNA by miRNAs could be an additional way to suppress IL6 translation in the presence of ongoing transcription. This would enable almost immediate translation after stimulation.

Last but not least, alignment of reference mRNA sequences using the UCSC Genome Browser (40) reveals a cis natural antisense transcript (cis-NAT) for IL6 with a 5' UTR overlap. CisNATs are antisense transcripts derived from transcription of the opposite DNA strand that overlap the sense transcript. Note that the promoter region of this antisense transcript is again highly conserved. Although the IL6 cis-NAT is listed in the recently released cis-NAT database, its functional or regulatory significance has not yet been investigated (89).

In contrast to IL6, where we already know that posttranscriptional regulation is a major factor contributing to its mRNA expression, the four mRNAs that code for paralogs of the HSPA1A gene located in proximity to each other on chromosome 6 do not show evidence for regulation by miRNA or recognition motives. Their promoter regions and UTRs are poorly conserved. Instead they are very short genes with 1-2 exons only. As shown in Figure 2a below"Human Chained Self Alignments" there are even more paralogs located in different chromosomal positions that are highly similar and do all code for HSP70 protein variants. This leads to the situation in which a protein can be generated quickly after stimulation by 1. transcriptional activation of two or more closely related HSPA1A genes or 2. by fast transcription of a small primary transcript (hnRNA). Interestingly, both HSPA1A coding gene and a number of other small genes are located within an intron of the RD RNA binding protein (RDBP). RDBP is known to be part of a multi-protein complex that negatively regulates the transcription of IEGs (89). It is possible that concomitant expression of RDBP and the intronic HSP70 variants occurs. If this is the case, it would lead to the situation that once the RDBP protein (long hnRNA) is translated, further transcription of HSP70 mRNA variants would be attenuated (1). As a consequence we would have a negative feedback loop, possibly important in preventing excessive production of HSP70 in those situations – such as during a marathon - where body temperature increases and is elevated for more than two hours. This leads to the prediction that the known down-regulation of HSP70 by regular endurance exercise (20, 24) may at least partially be mediated through elevated levels of RGDB.

Taken together IL6 mRNA and the HSP70 coding mRNAs appear to be two very different types of mRNAs known to be altered by exercise. While for

IL6 many different posttranscriptional mechanisms may act together to achieve expression in an IEG manner, the HSPA1A paralogs coding for HSP70 protein variants provide evidence for transcriptional regulation. Importantly, the diversity of transcriptional and posttranscriptional regulatory features shown for these two mRNAs has an impact on the detlectability of these candidates by different transcriptomic approaches and subsequent real-time RT-PCR.

The HSPA1A paralogs impose one main problem: Which of the paralogs or how many of them with what different kind of sensitivity for each one of them are we measuring with our procedure? When we apply real-time RT-PCR we could use primers that prime all of the different variants, specific primers that prime only one variant, or we could neglect this issue and just choose any kind of primer within one of the transcripts by chance. The estimates of expression will differ substantially. When we use short oligonucleotide microarrays for expression analysis like Connolly et al. (14) a similar problem may occur. These chips use so called mis-match probes, where just one base is exchanged compared to the perfect match probe. Mis-matches to one variant may accidentally be a good match for another HSP70 variant. Accordingly, the standard quantification software, MAS5, which takes match to mis-match detection ratios into account to calculate expression values, may end up with results that are relatively noisy in situations where a lot of paralogs are expressed.

It has already been shown that using the match signals only by applying the quantification procedure PDNN reduces measuring noise (88). Depending on the method used for data processing (MAS5, dChip PMMM, dChip PM, RMA, GC-RMA and PDNN) different genes will be identified as differentially regulated. In a recent study, there was only 27 to 36 % overlap of differentially regulated transcripts if the same raw data were processed by the different quantification procedures and differential regulation of most of the candidates could not be verified by real-time RT-PCR (49). In case cDNA microarrays are used for quantification the data processing procedure is not problematic, since there is in most cases only one probe to look at. Additionally, in the study of Fehrenbach and colleagues most of the candidates could be verified by real-time RT-PCR (25, 90). However, there is no evidence that the proportion of verifiable differentially regulated candidates is significantly higher in cDNA microarray studies than in studies using short oligonucleotide microarrays. Also a reduced false positive rate may come along with a severely reduced sensitivity.

Two questions arise when we consider the differential expression of IL6. 1. It has to be taken into account that there is a second reference mRNA for IL6 that has neither the known AREs nor the miRNA binding sites (Fig. 2b). It may well be that these two variants show differential regulation and all of the different quantification procedures may want to take this circumstance into account. 2. What happens with the detection ratio on a microarray or in a real-time PCR assay in those cases in which antisense sequences or miRNAs to the transcript are present? So far this issue has not yet been investigated to our knowledge. It must be considered that antisense or miRNAs could attenuate the binding of the target sequence to microarray probes and that standard RT-PCR can not differentiate whether a sense or an antisense sequence is amplified.

In addition to these pitfalls which apply to HSP70 or IL6 mRNA detec-

tion another problem is the regulation of translation by the polyA tail length of an mRNA or in principle, the polyA tail length difference between transcripts at all. Short oligonucleotide microarrays use T7 polymerase protocols to process and amplify the mRNA. The efficiency of this protocol may depend on the length of the polyA tail of the transcript. On the one hand only polyA positive RNA should be detectable with this method (64). On the other hand, 18s rRNA as a well known poly A negative RNA is used as a housekeeping gene by Affymetrix on various microarrays and expression values generally exceed those of most of the other transcripts. Therefore the influence of the polyA tail length on the expression level of a transcript determined by short oligonucleotide microarrays is not yet clear.

Conclusion

Different biophysical and humoral responses following exercise use common pathways to induce IEG expression profiles as described in Chapters 2 and 3 and illustrated in Figure 1. While the downstream cascades that follow IEG expression are highly complex interactive networks, IEG expression itself may only show limited variation in response patterns. Focusing studies on a level where we expect relatively low complexity is appropriate at the current stage of the technical procedures to study the transcriptome. An additional argument for focusing at the IEG level and therefore at the first line of transcriptional response is the increasing evidence for a much bigger size of the transcriptome and its fascinating interactivity. Future gene expression studies at the IEG level in exercise immunology may also contribute to unravel this interactivity and they will certainly help to bridge the gap between exercise and the induced phenotypic adaptation.

List of Abbreviations:

ARE	Adenosine- and Uracil-rich element
CArG box	CC(A/T)6GG box
cis-NAT	in cis natural antisense transcript
CPE	cytoplasmic polyadenylation element
eHSP	extracellular heat shock protein
hnRNA	heterogeneous nuclear RNA
HSP	heat shock protein
IEG	immediate early gene
miRNA	microRNA
ncRNA	non-coding RNA
PBMC	peripheral blood derived mononuclear cell
RNAi	RNA interference
TonE / ORE	tonicity / osmotic response element
UTR	untranslated region

List of genes mentioned:

AUF1 / HNRD	Heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA-binding protein 1, 37kD)
CD81	Cluster of differentiation 81
c-fos	FBJ murine osteosarcoma viral oncogene homolog
c-jun	Jun oncogene
EGR1	Early growth response gene 1
ERK	Elk-related tyrosine kinase
GSTM3	Glutathione S-transferase M3
HSPA1A	Heat shock 70kDa protein 1A
HSPA1B	Heat shock 70kDa protein 1B
HUR/ELAVL1	Hu antigen R; embryonic lethal, abnormal vision, drosophila, homolog-like 1
IL1-ra/IL1RN	Interleukin 1 receptor antagonist
IL2	Interleukin 2
IL6	Interleukin 6
IL8	Interleukin 8
JNK / MAPK8	c-Jun N-terminal kinase 1; mitogen-activated protein kinase 8
MAPKAP-K2	Mitogen-activated protein kinase-activated protein kinase 2
p38 / MAPK14	Mitogen-activated protein kinase 14; p38 mitogen activated protein kinase
PU1 / SPI1	spleen focus forming virus (SFFV) proviral; PU-box binding transcription factor 1
RDBP	RD RNA-binding protein
SAPK / MAPK9	stress-activated protein kinase
TMFα	Tumor necrosis factor α

Reference List

1. Aida, M., Y. Chen, K. Nakajima, Y. Yamaguchi, T. Wada, and H. Handa. Transcriptional pausing caused by NELF plays a dual role in regulating immediate-early expression of the junB gene. Mol Cell Biol. 26: 6094-6104, 2006.
2. Aranburu, A., D. Liberg, B. Honore, and T. Leanderson. CArG box-binding factor-A interacts with multiple motifs in immunoglobulin promoters and has a regulated subcellular distribution. Eur J Immunol. 36: 2192-2202, 2006.
3. Asea, A. Stress proteins and initiation of immune response: chaperokine activity of hsp72. Exerc.Immunol.Rev. 11: 34-45, 2005.
4. Bakheet, T., B. R. Williams, and K. S. Khabar. ARED 2.0: an update of AU-rich element mRNA database. Nucleic Acids Res 31: 421-423, 2003.
5. Barnard, D. C., K. Ryan, J. L. Manley, and J. D. Richter. Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. Cell 119: 641-651, 2004.
6. Campisi, J. and M. Fleshner. Role of extracellular HSP72 in acute stress-induced potentiation of innate immunity in active rats. J Appl Physiol 94: 43-52, 2003.

7. Cano, E. and L. C. Mahadevan. Parallel signal processing among mammalian MAPKs. Trends Biochem Sci. 20: 117-122, 1995.
8. Cano, E. and L. C. Mahadevan. Parallel signal processing among mammalian MAPKs. Trends Biochem Sci. 20: 117-122, 1995.
9. Carninci, P., T. Kasukawa, S. Katayama, et al. The transcriptional landscape of the mammalian genome. Science 309: 1559-1563, 2005.
10. Chtanova, T., R. A. Kemp, A. P. Sutherland, F. Ronchese, and C. R. Mackay. Gene microarrays reveal extensive differential gene expression in both CD4(+) and CD8(+) type 1 and type 2 T cells. J Immunol. 167: 3057-3063, 2001.
11. Claverie, J. M. Fewer genes, more noncoding RNA. Science 309: 1529-1530, 2005.
12. Clements, J. B., R. J. Watson, and N. M. Wilkie. Temporal regulation of herpes simplex virus type 1 transcription: location of transcripts on the viral genome. Cell 12: 275-285, 1977.
13. Cohen, D. M., J. C. Wasserman, and S. R. Gullans. Immediate early gene and HSP70 expression in hyperosmotic stress in MDCK cells. Am.J Physiol 261: C594-C601, 1991.
14. Connolly, P. H., V. J. Caiozzo, F. Zaldivar, D. Nemet, J. Larson, S. P. Hung, J. D. Heck, G. W. Hatfield, and D. M. Cooper. Effects of exercise on gene expression in human peripheral blood mononuclear cells. J Appl Physiol 97: 1461-1469, 2004.
15. Curran, T., W. P. MacConnell, F. van Straaten, and I. M. Verma. Structure of the FBJ murine osteosarcoma virus genome: molecular cloning of its associated helper virus and the cellular homolog of the v-fos gene from mouse and human cells. Mol Cell Biol. 3: 914-921, 1983.
16. Datta, R., N. Taneja, V. P. Sukhatme, S. A. Qureshi, R. Weichselbaum, and D. W. Kufe. Reactive oxygen intermediates target CC(A/T)6GG sequences to mediate activation of the early growth response 1 transcription factor gene by ionizing radiation. Proc.Natl.Acad.Sci.U.S.A 90: 2419-2422, 1993.
17. DeMaria, C. T. and G. Brewer. AUF1 binding affinity to A+U-rich elements correlates with rapid mRNA degradation. J Biol.Chem. 271: 12179-12184, 1996.
18. Denhardt, D. T. Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signalling. Biochem J 318 (Pt 3): 729-747, 1996.
19. Fehrenbach, E. and A. M. Niess. Role of heat shock proteins in the exercise response. Exerc.Immunol.Rev. 5: 57-77, 1999.
20. Fehrenbach, E., A. M. Niess, R. Veith, H. H. Dickhuth, and H. Northoff. Changes of HSP72-expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature. J.Leukoc.Biol. 69: 747-754, 2001.
21. Fehrenbach, E., A. M. Niess, K. Voelker, H. Northoff, and F. C. Mooren. Exercise intensity and duration affect blood soluble HSP72. Int.J Sports Med. 26: 552-557, 2005.
22. Fehrenbach, E. and H. Northoff. Free radicals, exercise, apoptosis, and heat shock proteins. Exerc.Immunol.Rev. 7: 66-89, 2001.
23. Fehrenbach, E. and H. Northoff. Free radicals, exercise, apoptosis, and heat shock proteins. Exerc.Immunol.Rev. 7: 66-89, 2001.
24. Fehrenbach, E., F. Passek, A. M. Niess, H. Pohla, C. Weinstock, H. H. Dickhuth, and H. Northoff. HSP expression in human leukocytes is modulated by endurance exercise. Med.Sci.Sports Exerc. 32: 592-600, 2000.

25. Fehrenbach, E., D. Zieker, A. M. Niess, E. Moeller, S. Russwurm, and H. Northoff. Microarray technology--the future analyses tool in exercise physiology? Exerc.Immunol.Rev. 9: 58-69, 2003.
26. Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391: 806-811, 1998.
27. Fleshner, M., J. Campisi, and J. D. Johnson. Can exercise stress facilitate innate immunity? A functional role for stress-induced extracellular Hsp72. Exerc.Immunol.Rev. 9: 6-24, 2003.
28. Godfrey, R. J., Z. Madgwick, and G. P. Whyte. The exercise-induced growth hormone response in athletes. Sports Med. 33: 599-613, 2003.
29. Grun, D., Y. L. Wang, D. Langenberger, K. C. Gunsalus, and N. Rajewsky. microRNA target predictions across seven Drosophila species and comparison to mammalian targets. PLoS Comput.Biol. 1: e13, 2005.
30. Han, J., J. D. Lee, L. Bibbs, and R. J. Ulevitch. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science 265: 808-811, 1994.
31. Hashimoto, S., S. Nagai, J. Sese, T. Suzuki, A. Obata, T. Sato, N. Toyoda, H. Y. Dong, M. Kurachi, T. Nagahata, K. Shizuno, S. Morishita, and K. Matsushima. Gene expression profile in human leukocytes. Blood 101: 3509-3513, 2003.
32. Hazzalin, C. A., R. Le Panse, E. Cano, and L. C. Mahadevan. Anisomycin selectively desensitizes signalling components involved in stress kinase activation and fos and jun induction. Mol Cell Biol. 18: 1844-1854, 1998.
33. He, H., C. Chen, Y. Xie, A. Asea, and S. K. Calderwood. HSP70 and heat shock factor 1 cooperate to repress Ras-induced transcriptional activation of the c-fos gene. Cell Stress.Chaperones. 5: 406-411, 2000.
34. Heo, J. I., M. S. Lee, J. H. Kim, J. S. Lee, J. Kim, J. B. Park, J. Y. Lee, J. A. Han, and J. I. Kim. The role of tonicity responsive enhancer sites in the transcriptional regulation of human hsp70-2 in response to hypertonic stress. Exp.Mol Med. 38: 295-301, 2006.
35. Herbert, A. The four Rs of RNA-directed evolution. Nat.Genet. 36: 19-25, 2004.
36. Hoffmann, J., P. Simon, A. K. Zimmermann, M. Lemancyk, T. Walter, M. Beyer, H. M. Hoffmeister, G. Ziemer, and H. P. Wendel. Thrombospondin 1 as possible key factor in the hemocompatibility of endocoronary prostheses. Biomaterials 26: 5240-5250, 2005.
37. Ingber, D. E. Cellular mechanotransduction: putting all the pieces together again. FASEB J 20: 811-827, 2006.
38. Karin, M. Signal transduction from the cell surface to the nucleus through the phosphorylation of transcription factors. Curr.Opin.Cell Biol. 6: 415-424, 1994.
39. Katayama, S., Y. Tomaru, T. Kasukawa, et al. Antisense transcription in the mammalian transcriptome. Science 309: 1564-1566, 2005.
40. Kent, W. J., C. W. Sugnet, T. S. Furey, K. M. Roskin, T. H. Pringle, A. M. Zahler, and D. Haussler. The human genome browser at UCSC. Genome Res 12: 996-1006, 2002.
41. Kojima, R., J. D. Randall, E. Ito, H. Manshio, Y. Suzuki, and S. R. Gullans. Regulation of expression of the stress response gene, Osp94: identification of the tonicity response element and intracellular signalling pathways. Biochem J 380: 783-794, 2004.
42. Krek, A., D. Grun, M. N. Poy, R. Wolf, L. Rosenberg, E. J. Epstein, P. MacMenamin, P. da, I, K. C. Gunsalus, M. Stoffel, and N. Rajewsky. Combinatorial

microRNA target predictions. Nat.Genet. 37: 495-500, 2005.
43. Lancaster, G. I. and M. A. Febbraio. Mechanisms of stress-induced cellular HSP72 release: implications for exercise-induced increases in extracellular HSP72. Exerc.Immunol.Rev. 11: 46-52, 2005.
44. Lopez, d. S., I, M. Zhan, A. Lal, X. Yang, and M. Gorospe. Identification of a target RNA motif for RNA-binding protein HuR. Proc.Natl.Acad.Sci.U.S.A 101: 2987-2992, 2004.
45. Marshall, H. C., R. A. Ferguson, and M. A. Nimmo. Human resting extracellular heat shock protein 72 concentration decreases during the initial adaptation to exercise in a hot, humid environment. Cell Stress.Chaperones. 11: 129-134, 2006.
46. Mattick, J. S. The functional genomics of noncoding RNA. Science 309: 1527-1528, 2005.
47. McCormick, C. and D. Ganem. The kaposin B protein of KSHV activates the p38/MK2 pathway and stabilizes cytokine mRNAs. Science 307: 739-741, 2005.
48. Meissner, A., I. Luss, N. Rolf, P. Boknik, U. Kirchhefer, V. Kehm, J. Knapp, B. Linck, H. Luss, F. U. Muller, T. Weber, W. Schmitz, H. Van Aken, and J. Neumann. The early response genes c-jun and HSP-70 are induced in regional cardiac stunning in conscious mammals. J Thorac.Cardiovasc Surg. 119: 820-825, 2000.
49. Millenaar, F. F., J. Okyere, S. T. May, M. van Zanten, L. A. Voesenek, and A. J. Peeters. How to decide? Different methods of calculating gene expression from short oligonucleotide array data will give different results. BMC.Bioinformatics. 7: 137, 2006.
50. Miller, A. D., T. Curran, and I. M. Verma. c-fos protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. Cell 36: 51-60, 1984.
51. Moldoveanu, A. I., R. J. Shephard, and P. N. Shek. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. J Appl Physiol 89: 1499-1504, 2000.
52. Moore, M. J. From birth to death: the complex lives of eukaryotic mRNAs. Science 309: 1514-1518, 2005.
53. Mooren, F. C. and K. Volker. Exercise-induced modulation of intracellular signaling pathways. Exerc.Immunol.Rev. 7: 32-65, 2001.
54. Morgan, J. I. and T. Curran. Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. Trends Neurosci. 12: 459-462, 1989.
55. Niess, A. M., H. H. Dickhuth, H. Northoff, and E. Fehrenbach. Free radicals and oxidative stress in exercise--immunological aspects. Exerc.Immunol.Rev. 5: 22-56, 1999.
56. Palmer, C., M. Diehn, A. A. Alizadeh, and P. O. Brown. Cell-type specific gene expression profiles of leukocytes in human peripheral blood. BMC.Genomics 7: 115, 2006.
57. Paris, J. and J. D. Richter. Maturation-specific polyadenylation and translational control: diversity of cytoplasmic polyadenylation elements, influence of poly(A) tail size, and formation of stable polyadenylation complexes. Mol Cell Biol. 10: 5634-5645, 1990.
58. Peng, S. S., C. Y. Chen, N. Xu, and A. B. Shyu. RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. EMBO J 17: 3461-3470, 1998.
59. Pillai, R. S., S. N. Bhattacharyya, C. G. Artus, T. Zoller, N. Cougot, E. Basyuk, E. Bertrand, and W. Filipowicz. Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science 309: 1573-1576, 2005.

60. Pirkkala, L., P. Nykanen, and L. Sistonen. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J 15: 1118-1131, 2001.
61. Radons, J. and G. Multhoff. Immunostimulatory functions of membrane-bound and exported heat shock protein 70. Exerc.Immunol.Rev. 11: 17-33, 2005.
62. Roizman, B., M. Kozak, R. W. Honess, and G. Hayward. Regulation of herpesvirus macromolecular synthesis: evidence for multilevel regulation of herpes simplex 1 RNA and protein synthesis. Cold Spring Harb.Symp.Quant.Biol. 39 Pt 2: 687-701, 1975.
63. Rouse, J., P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt, and A. R. Nebreda. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. Cell 78: 1027-1037, 1994.
64. Schneider, J., A. Buness, W. Huber, J. Volz, P. Kioschis, M. Hafner, A. Poustka, and H. Sultmann. Systematic analysis of T7 RNA polymerase based in vitro linear RNA amplification for use in microarray experiments. BMC.Genomics 5: 29, 2004.
65. Seger, R. and E. G. Krebs. The MAPK signaling cascade. FASEB J 9: 726-735, 1995.
66. Soloaga, A., S. Thomson, G. R. Wiggin, N. Rampersaud, M. H. Dyson, C. A. Hazzalin, L. C. Mahadevan, and J. S. Arthur. MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. EMBO J 22: 2788-2797, 2003.
67. Steensberg, A. The role of IL-6 in exercise-induced immune changes and metabolism. Exerc.Immunol.Rev. 9: 40-47, 2003.
68. Stringer, J. R., L. E. Holland, and E. K. Wagner. Mapping early transcripts of herpes simplex virus type 1 by electron microscopy. J Virol. 27: 56-73, 1978.
69. Stula, M., H. D. Orzechowski, S. Gschwend, R. Vetter, R. von Harsdorf, R. Dietz, and M. Paul. Influence of sustained mechanical stress on Egr-1 mRNA expression in cultured human endothelial cells. Mol Cell Biochem 210: 101-108, 2000.
70. Sukhatme, V. P., X. M. Cao, L. C. Chang, C. H. Tsai-Morris, D. Stamenkovich, P. C. Ferreira, D. R. Cohen, S. A. Edwards, T. B. Shows, T. Curran, and . A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. Cell 53: 37-43, 1988.
71. Suzuki, K., S. Nakaji, S. Kurakake, M. Totsuka, K. Sato, T. Kuriyama, H. Fujimoto, K. Shibusawa, K. Machida, and K. Sugawara. Exhaustive exercise and type-1/type-2 cytokine balance with special focus on interleukin-12 p40/p70. Exerc.Immunol.Rev. 9: 48-57, 2003.
72. Suzuki, K., S. Nakaji, M. Yamada, M. Totsuka, K. Sato, and K. Sugawara. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc.Immunol.Rev. 8: 6-48, 2002.
73. Thomson, S., A. L. Clayton, C. A. Hazzalin, S. Rose, M. J. Barratt, and L. C. Mahadevan. The nucleosomal response associated with immediate-early gene induction is mediated via alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG-14 kinase. EMBO J 18: 4779-4793, 1999.
74. Treisman, R. Regulation of transcription by MAP kinase cascades. Curr.Opin.Cell Biol. 8: 205-215, 1996.
75. van Straaten, F., R. Muller, T. Curran, C. Van Beveren, and I. M. Verma. Complete nucleotide sequence of a human c-onc gene: deduced amino acid sequence

of the human c-fos protein. Proc.Natl.Acad.Sci.U.S.A 80: 3183-3187, 1983.
76. Vaughn, M. W. and R. Martienssen. It's a small RNA world, after all. Science 309: 1525-1526, 2005.
77. Waldegger, S. and F. Lang. Cell volume and gene expression. J Membr.Biol. 162: 95-100, 1998.
78. Watson, R. J. and J. B. Clements. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. Nature 285: 329-330, 1980.
79. Whitmarsh, A. J. and R. J. Davis. Structural organization of MAP-kinase signaling modules by scaffold proteins in yeast and mammals. Trends Biochem Sci. 23: 481-485, 1998.
80. Wiggin, G. R., A. Soloaga, J. M. Foster, V. Murray-Tait, P. Cohen, and J. S. Arthur. MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. Mol Cell Biol. 22: 2871-2881, 2002.
81. Willingham, A. T., A. P. Orth, S. Batalov, E. C. Peters, B. G. Wen, P. Aza-Blanc, J. B. Hogenesch, and P. G. Schultz. A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. Science 309: 1570-1573, 2005.
82. Wilusz, C. J., M. Wormington, and S. W. Peltz. The cap-to-tail guide to mRNA turnover. Nat.Rev.Mol Cell Biol. 2: 237-246, 2001.
83. Winzen, R., M. Kracht, B. Ritter, A. Wilhelm, C. Y. Chen, A. B. Shyu, M. Muller, M. Gaestel, K. Resch, and H. Holtmann. The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. EMBO J 18: 4969-4980, 1999.
84. Yamaguchi, S., M. Yamaguchi, E. Yatsuyanagi, S. S. Yun, N. Nakajima, J. A. Madri, and B. E. Sumpio. Cyclic strain stimulates early growth response gene product 1-mediated expression of membrane type 1 matrix metalloproteinase in endothelium. Lab Invest 82: 949-956, 2002.
85. Yang, J. H., H. Sakamoto, E. C. Xu, and R. T. Lee. Biomechanical regulation of human monocyte/macrophage molecular function. Am.J Pathol 156: 1797-1804, 2000.
86. Yu, M., E. Blomstrand, A. V. Chibalin, A. Krook, and J. R. Zierath. Marathon running increases ERK1/2 and p38 MAP kinase signalling to downstream targets in human skeletal muscle. J Physiol 536: 273-282, 2001.
87. Zamore, P. D. and B. Haley. Ribo-gnome: the big world of small RNAs. Science 309: 1519-1524, 2005.
88. Zhang, L., M. F. Miles, and K. D. Aldape. A model of molecular interactions on short oligonucleotide microarrays. Nat.Biotechnol. 21: 818-821, 2003.
89. Zhang, Y., X. S. Liu, Q. R. Liu, and L. Wei. Genome-wide in silico identification and analysis of cis natural antisense transcripts (cis-NATs) in ten species. Nucleic Acids Res 34: 3465-3475, 2006.
90. Zieker, D., E. Fehrenbach, J. Dietzsch, J. Fliegner, M. Weidmann, K. Nieselt, P. Gebicke-Haerter, R. Spanagel, P. Simon, A. M. Niess, and H. Northoff. cDNA-microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. Physiol Genomics 2005.
91. Zieker, D., J. Zieker, J. Dietzsch, M. Burnet, H. Northoff, and E. Fehrenbach. CDNA-microarray analysis as a research tool for expression profiling in human peripheral blood following exercise. Exerc.Immunol.Rev. 11: 86-96, 2005.